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Randomized Phase II Trial of Rubidazone and Adriamycin in Women With Advanced Breast Cancer [1,2]

James N. Ingle,* David L. Ahmann, Judith R. O'Fallon, Harry F. Bisel, Joseph Rubin, Larry K. Kvols, and Emilio R. Giuliani [3,4]

SUMMARY

Thirty-eight females with advanced breast cancer who had failed prior chemotherapy were entered in a randomized study of rubidazone (150 mg/m² iv over 1 hour) versus Adriamycin (60 mg/m² iv over 5 minutes), both given every 4 weeks. The two treatment groups each contained 19 patients and were similar with respect to age, menopausal status, dominant disease status, Eastern Cooperative Oncology Group performance score, and prior hormonal therapy and chemotherapy exposure. No regressions (complete or partial) were observed with rubidazone, but four (21%) patients achieved regressions (one complete and three partial) with Adriamycin. Considering all cycles, hematologic toxicity included leukopenia (wbc count nadir of < 4000/mm³) in 88% of patients receiving rubidazone and in 89% of those receiving Adriamycin. Three of eight patients treated with Adriamycin after rubidazone failure achieved a regression. Rubidazone appears to be of little value in the treatment of patients with advanced breast cancer who have failed prior chemotherapy.

[Cancer Treat Rep 63:1701–1705, 1979]

Adriamycin is considered to be the most active single agent in the treatment of advanced breast cancer. A review of five series revealed a cumulative response rate of 43% (54 of 126 patients) for Adriamycin used alone in patients with no prior chemotherapy (1). A randomized study of Adriamycin versus multiple-drug programs (cyclophosphamide, 5-fluorouracil, and prednisone with or without vincristine) in patients with no previous chemotherapy exposure showed response rates of 50% (ten of 20 patients) and 46% (13 of 28 patients), respectively (2). The median durations of remission in this study were 32 weeks for Adriamycin and 40 and 42 weeks for the multiple-drug programs. As secondary treatment after failure with combination chemotherapy, Adriamycin has produced response rates of 20% (ten of 49 patients) (3) and 11% (one of nine patients) (2). The major dose-limiting toxicity of Adriamycin is cardiotoxicity which is manifested by a clinical syndrome of congestive heart failure (4).

Rubidazone, the benzoylhydrazone derivative of daunorubicin, is active in several experimental rodent tumor systems including L1210 leukemia, P388 lymphocytic leukemia, and ip B16 melanoma.[5] Zbinden and Brändle (5) studied the relative cardiotoxicity of various anthracycline analogs in vivo in female rats and it appeared that rubidazone was less cardiotoxic than Adriamycin and daunorubicin on a milligram per meter squared basis. A phase I trial of rubidazone was performed in patients with advanced solid tumors by Kovach et al (6) who recommended a dose level of 150 mg/m² every 3–4 weeks.

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[4]We gratefully acknowledge the assistance of Ann Fehrenkamp in data processing.

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[5]Henney JE, Von Hoff DD, Penta J, et al. Clinical brochure: rubidazone (NSC-164011). Prepared by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, Nov 1976.

Rubidazone has been shown to be active in patients with hematologic malignancies. Jacquillat et al (7) reported a complete remission rate of 57% (40 of 70 patients) in previously untreated patients with acute myelocytic leukemia. A phase I and II trial of rubidazone by Benjamin et al (8) produced a 33% complete remission rate in 39 previously treated adults with acute leukemia and they concluded that it was the most active single antileukemic agent they had tested.

Less data exist on the value of rubidazone in solid tumors. Skovsgaard et al (9) treated 24 patients with solid tumors with rubidazone at one of three dose levels (100, 150, or 200 mg/m^2) and saw no objective responses (complete or partial). Their study group included seven patients with breast cancer, all of whom had received prior treatment with surgery, radiation therapy, and chemotherapy (unspecified). Chauvergne et al (10) recently reported that three of 21 patients with breast cancer had at least a 50% reduction in tumor volume with rubidazone therapy.

The need for the development of new agents with an improved therapeutic index in breast cancer is clear. The discovery of an anthracycline analog with less cardiotoxicity than Adriamycin and with greater or even similar therapeutic efficacy would potentially be of significant benefit to patients with a variety of neoplastic diseases in addition to breast cancer. On this basis, rubidazone was selected for study. We utilized a randomization between rubidazone and Adriamycin in order to have an "internal control" for evaluation of efficacy as well as a potential means for determining relative cardiotoxicity should rubidazone prove to be effective therapy.

MATERIALS AND METHODS

Thirty-eight females with histologically confirmed breast cancer and progressive metastatic disease were entered in the study. Eligibility requirements included failure with a prior chemotherapy program, no previous treatment with an anthracycline, and measurable disease. Patients were required to have a wbc count of $\geq$ 4000/mm^3, a platelet count of $\geq$ 100,000/mm^3, a total bilirubin level of $\leq$ 1.5 mg/dl, a creatinine of $\leq$ 3.0 mg/100 ml, and an Eastern Cooperative Oncology Group (ECOG) performance score of $\leq$ 3. Patients with active cardiac disease defined as congestive heart failure, significant arrhythmia, bilateral bundle branch block, or history of myocardial infarction within the previous 4 months were not eligible for participation in the study. Those patients who failed with estrogen therapy after an initial response must have had the estrogen discontinued for at least 4 weeks with progression in the interim.

Studies obtained prior to initial therapy consisted of medical history, physical examination with measurement of indicator lesions, hemoglobin, wbc and platelet counts, chemistry profile (sodium, potassium, calcium, phosphorus, total protein, glucose, alkaline phosphatase, SGOT, total and direct bilirubin, uric acid, and creatinine), chest roentgenogram, metastatic bone survey (skull, cervical, thoracic, lumbar spines, and pelvis), ECG, and phonoechocardiogram. All studies were repeated prior to each subsequent treatment except for the metastatic bone survey which was performed every 3 months. Patients were stratified according to (*a*) ECOG performance score (0–1 versus 2–3), (*b*) dominant disease status (soft tissue versus osseous versus visceral), and (*c*) age (< 35 years versus 35–50 years versus > 50 years) and were then randomized to receive treatment with either Adriamycin or rubidazone. Adriamycin was administered at a dose of 60 mg/m^2 iv over 5 minutes and rubidazone was administered at a dose of 150 mg/m^2 by iv infusion over 1 hour. Patients being treated with rubidazone received Benadryl (50 mg iv) prior to initiation of the infusion. Following each chemotherapy treatment, interval wbc and platelet counts were obtained once a week and were used to make dose modifications in subsequent cycles to ensure adequate myelosuppression (ie, wbc count nadir of 1500–3000/mm^3).

All patients were re-assessed every 28 days. Treatment was continued providing the patient's status was that of stable disease or disease regression (complete or partial). A complete regression (CR) was defined as the disappearance of all evidence of tumor. A partial regression (PR) was defined as a $\geq$ 50% reduction in the sum of the products of the perpendicular diameters of marker lesions. There must have been no progression in any lesion or appearance of new lesions. In the case of hepatomegaly, there must have been a reduction by one third in the sum of the linear measurements below each costal margin in the midclavicular line and the xiphoid. In the case of osseous metastases, roentgenographic evidence of healing, ie, re-ossification of previously lytic lesions, was considered a PR. Progressive disease was defined as $\geq$ 25% increase in the product of the perpendicular diameters of measurable lesions, the appearance of any new lesion, or the decline of two or more levels on the ECOG scale not explained by non-tumor-related illness. Stability was defined as the failure to qualify for either a regression (CR or PR) or progressive disease. Duration of regression was measured from Day 1 of treatment to first observation of progressive disease in those patients who achieved a regression, and interval to progression was measured from Day 1 of treatment to first observation of progressive disease. A patient was considered to be evaluable if she received at least Day 1 of therapy and returned for one evaluation or, in the case of her inability to return, she was considered to have disease progression by her local physician. Patients who died prior to a first re-assessment were considered evaluable unless death was unequivocally due to a non-tumor- or

TABLE 1.—Patient characteristics of both treatment groups

	No. of patients	
	Adriamycin	Rubidazone
Total	19	19
Age (yrs)		
35–50	6	5
> 50	13	14
(Median: range)	(60: 36–73)	(57: 35–80)
Menopausal status		
Premenopausal	2	2
Postmenopausal	17	17
Natural (yrs)	13	14
1–5	1	2
> 5–10	4	3
> 10	8	9
Surgical castration	3	2
Radiation castration	1	1
Disease-free interval (yrs)		
< 1	3 (16%)	8 (42%)
1–3	8 (42%)	9 (47%)
> 3–5	5 (26%)	2 (11%)
> 5	3 (15%)	0
Dominant disease status		
Soft tissue	3	2
Osseous	4	4
Visceral	12	13
ECOG performance score		
0–1	12	13
2–3	7	6
Prior additive hormonal therapy	11	12
Prior chemotherapy*		
CFP	12	9
CFP + MER-BCG	4	6
Melphalan, CFP	2	3
5-FU	1	1

*CFP = cyclophosphamide, 5-fluorouracil (5-FU), and prednisone; MER-BCG = methanol-extracted residue of bacillus Calmette-Guérin.

non-treatment-related cause. Patients who developed progressive disease were followed for survival.

Patient Characteristics

All 38 patients entered in the study were evaluable and were equally divided between the Adriamycin group and the rubidazone group. Similarities in patient characteristics are seen between the two groups (table 1) with respect to age, menopausal status, dominant disease status, ECOG performance score, and prior hormonal therapy and chemotherapy. The two treatment groups had significantly different distributions of disease-free interval (median test, $P < 0.01$). The percentages of patients with disease-free intervals of < 20 months (the median for all study patients) were 74% for the rubidazone group and 26% for the Adriamycin group.

RESULTS

Response Data

Response data for the two groups are given in table 2. No regressions were observed with rubidazone. The fact that no regressions were observed in 19 consecutive patients enables us to conclude, with 95% certainty, that the true regression probability for rubidazone is < 15%. Four patients had objective regressions after Adriamycin treatment. There was one CR (indicator site: liver) with a duration of 368 days and three PRs (indicator sites: liver, liver, and soft tissue) with durations of 127, 131, and 370 days, respectively. Despite the small size of the treatment groups, the difference in observed regression rates between Adriamycin and rubidazone approached statistical significance (chi-square test, $0.05 < P < 0.10$).

Although not called for in the protocol, eight patients were treated with Adriamycin (in a nonstudy setting) after failure with rubidazone. Three of these patients achieved a PR.

Time to Progression

The median times to progression were 56 days for the rubidazone group and 84 days for the Adriamycin group. Analysis of the distribution of time to progression revealed it to be somewhat worse for the rubidazone group, although not significantly so (log-rank, $P = 0.088$).

A Cox co-variate analysis (11) was performed to assess the degree of association between time to progression and certain prognostic factors (treatment, age, pretreatment performance score, dominant disease status, and disease-free interval). Only one of these factors proved to be even marginally associated with time to progression, and that was treatment ($P = 0.08$).

Survival

The median survival is in the range of 249–263 days for the rubidazone group and 225 days for the Adriamycin group. There is no significant difference in the survival distributions between the two treatment groups (log-rank, $P = 0.746$).

Toxicity

Myelosuppression (wbcs < 4000/mm^3 and platelets < 100,000/mm^3) was the most common side effect encountered and was similar for the two treatment groups (table 3). No drug-related sepsis or bleeding was encountered. Nonhematologic toxicity was mild. Despite pretreatment of patients receiving rubidazone with iv Benadryl, three patients developed mild urticaria. No patient developed signs or symptoms of cardiotoxicity while in the study. However, the number of treatment cycles with rubida-

TABLE 2.—Response data

	Adriamycin		Rubidazone	
	No.	%	No.	%
Patients entered	19	100	19	100
Best response achieved				
CR	1	5	0	—
PR	3	16	0	—
Stable	12	63	12	63
Progression (immediate)	3	16	7	37
Total progressions	19	100	19	100
Time to progression (days)				
Median	84		56	
Range	12–370		5–209	
Total patients dead	14	74	13	68
Survival (days)				
Median	225		249–263	
Range	25–542*		18–567*	

*Continues in effect.

TABLE 3.—Toxicity

	Adriamycin	Rubidazone
Hematologic toxicity		
No. of evaluable patients	18	17
Cycle 1		
No. of patients with—		
Leukopenia*	15 (83%)	13 (76%)
Wbc count nadir (cells/mm^3)†		
Median	1800	2200
Range	1100–3600	700–3100
Thrombocytopenia‡	0	3 (18%)
Platelet count nadirs (cells/mm^3)		8000, 37,000, 94,000
All cycles		
No. of patients with—		
Leukopenia*	16 (89%)	15 (88%)
Wbc count nadir (cells/mm^3)†		
Median	1550	2300
Range	500–3400	700–3000
Thrombocytopenia‡	2 (11%)	3 (18%)
Platelet count nadirs (cells/mm^3)	23,000, 91,000	8000, 37,000 77,000
Nonhematologic toxicity		
No. of evaluable patients	18	18
No. of patients with—		
Nausea	11 (61%)	7 (39%)
Vomiting	8 (44%)	3 (17%)
Stomatitis	4 (22%)	3 (17%)
Urticaria	0	3 (17%)

*Wbc count of < 4000/mm^3.
†For patients developing leukopenia.
‡Platelet count of < 100,000/mm^3.

zone was small for most patients because of the lack of therapeutic efficacy: seven patients received one cycle, seven received two cycles, three received three cycles, one received four cycles, and one received seven cycles. E. R. Giuliani reviewed all of the ECGs and phonoechocardiograms but there were insufficient data to determine the cardiotoxicity of rubidazone or the relative cardiotoxicity of the two agents.

One patient treated with Adriamycin after failure with rubidazone developed congestive heart failure and died shortly thereafter. She had received 449 mg/m^2 of Adriamycin over eight monthly cycles and 460 mg/m^2 of rubidazone over three monthly cycles.

DISCUSSION

No objective regressions were observed in the 19 patients treated with rubidazone. The conclusion that rubidazone does not have appreciable activity in patients with advanced breast cancer who have failed prior chemotherapy (95% certainty that the true regression probability is $< 15\%$) is strengthened by the fact that it was assessed in the setting of a prospective randomized trial in which Adriamycin produced a 21% objective regression rate. The regression rate for Adriamycin observed in this study is consistent with the 20% rate reported by Hoogstraten et al (3) in patients with prior combination-chemotherapy exposure. The characteristics of the two treatment groups were comparable except for a tendency for shorter disease-free intervals in the rubidazone group. Co-variate analysis failed to show a significant association between disease-free interval and time to progression in these 38 patients. Although not a part of the study design, the fact that regressions were seen in three of eight patients treated with Adriamycin after failure with rubidazone further suggests that rubidazone is of little value as utilized in this study.

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Treatment of Grade III and IV Astrocytomas With BCNU Alone and in Combination With VM-26 Following Surgery and Radiation Therapy [1,2]

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SUMMARY

Twenty-one patients with grade III or IV astrocytomas were assigned randomly to receive either BCNU alone or BCNU and VM-26 after surgery and radiation therapy. Patients surviving radiation therapy and receiving single-agent chemotherapy had a median survival of 14 months while those receiving combination chemotherapy had a median survival of 22 months ($P > 0.05$). None of the patients who failed BCNU-only therapy responded to VM-26. Performance status was not affected by either regimen. Computerized tomographic scanning of the brain was useful only in confirming tumor progression. Two patients died from BCNU-related interstitial pulmonary fibrosis.

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Patients with highly malignant glial tumors such as grade III and IV astrocytomas (glioblastoma multiforme) live only 2–4 months from the time of diagnosis with no intervention (1). Megavoltage radiation therapy following surgery may lengthen survival to 9 months (2). The chemotherapeutic approach to the management of primary glial brain tumors yielded disappointing results until the development of nitrosourea compounds (2–5). This group of compounds demonstrates both alkylating and carbamoylation activity. In particular, the nitrosoureas are lipid-soluble and of low molecular weight, properties which are required to penetrate the "blood-brain barrier." The most active of the nitrosourea compounds against primary brain tumors is BCNU, which is capable of delaying disease progression in 40%–50% of malignant gliomas and may prolong median survival to 15 months (5,6). Recently, VM-26, a semisynthetic podophyllotoxin derivative, has been shown to be particularly effective in some patients with malignant gliomas (7,8). VM-26 is a spindle poison of high lipid solubility and low molecular weight. VM-26 has not had extensive testing in combination with nitrosoureas for primary central nervous system tumors (9). The purpose of this study was to compare the effect of BCNU alone versus the combination of BCNU and VM-26 on survival in patients with grade III or IV astrocytomas after surgery and radiation therapy.

PATIENTS AND METHODS

Thirty-one patients were entered in the study between January 1975 and July 1976. Ten of these patients died prior to the completion of radiation therapy. Eligibility for the study included patients with grade III or IV astrocytoma (glioblastoma multiforme) substantiated by histologic review (cytologic examination of cerebrospinal fluid was not considered an adequate technique for establishing the diagnosis). Three patients with surgically inaccessible tumors, but fulfilling the usual criteria for primary brain tumor by neurologic deficits and contrast studies, were considered for the study. Patients with pineal tumors were not included. Patient evaluation included a medical history and physical examination, chest roentgenogram, ECG, routine blood chemistries, and urinalysis. All patients underwent computerized axial

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TABLE 1.—Hematologic toxicity: BCNU dose modification schedule

Wbc count (cells/mm³)		Platelet count (cells/mm³)	Dose of BCNU
> 4000		> 100,000	100%
3000–4000	or	75,000–100,000	75%
2000–2999	or	50,000–74,999	50%
< 2000	or	< 50,000	None

tomographic (CAT) brain scan, cerebral arteriography, and, when indicated, pneumoencephalography. Patient performance status was graded just prior to the chemotherapy using the Eastern Cooperative Oncology Group criteria of 0 (fully active), 1 (ambulatory, capable of light work), 2 (in bed < 50% of time and capable of self-care), 3 (in bed or chair > 50% of time and capable of only limited self-care), and 4 (completely disabled). Performance status is used only as a semiquantitative measure of the quality of life. The usefulness and sensitivity of the CAT scan in evaluating responsiveness to chemotherapy as used in this study will be the subject of another report.

After surgery or diagnosis, all patients received 4000 rads of megavoltage whole-brain irradiation followed by an additional 2000 rads to the local tumor using CAT scan for accurate determination of tumor size and location. In most patients, chemotherapy was begun 2 weeks after the completion of radiation therapy. Patients were randomized, with their consent, to receive either BCNU alone (75 mg/m² iv on Days 1 and 2, repeated every 4 weeks) (group I) or BCNU (75 mg/m² iv on Days 1 and 2) followed by VM-26 (100 mg/m² iv on Days 29, 36, and 43) (group II). The cycle was repeated every 7 weeks. Group I failures were given VM-26 at a dose of 100 mg/m² iv on Days 1, 8, 15, 22, 29, and 36; the cycle was repeated every 7 weeks. A dose modification employed for BCNU hematologic toxicity is shown in table 1. Decadron was given to some patients throughout their illness and to all patients for brief periods of time. It is assumed that this corticosteroid has no direct antitumor effect and acts only on associated cerebral edema. Chemotherapy was continued until death or failure as indicated by CAT scan. Long-term survivors received chemotherapy for 2 years.

Followup evaluation included weekly blood cell counts and a physical examination and liver and renal function tests every 6 weeks. CAT brain scanning was performed upon the completion of radiation therapy and every 3 months thereafter. Patients who demonstrated neurologic deterioration underwent more frequent CAT examination.

TABLE 2.—Patient characteristics in groups I and II

Characteristic	Group I: BCNU alone (11 patients)	Group II: BCNU + VM-26 (10 patients)
Median age in yrs (range)	38 (8–62)	29 (12–64)
No. of males/females	4/7	7/3
No. of patients with—		
Tumor grade III/IV (when known)	5/3	6/3
Locations of primary tumor		
Frontal	1	2
Parietal	6	4
Temporal	0	2
Cerebellum	1	1
Thalamus	2	1
Pons	1	0
Median initial performance status	2	2
Median survival in mos	14	22

RESULTS

Patient characteristics in groups I and II are compared in table 2. The median time from diagnosis to initiation of chemotherapy was 14 weeks. Eleven patients received BCNU alone and ten patients received BCNU and VM-26; seven patients who were considered to be BCNU failures received VM-26. Among the patients treated with BCNU alone (group I), four were males and seven were females, with a median age of 38 years (range, 8–62). Five of these group I patients had grade III astrocytomas and three had grade IV astrocytomas. One patient had a thalamic tumor and another patient had a pontine glioma diagnosed by clinical and radiographic studies but not confirmed histologically. One patient with a parietal mass underwent biopsy but the sample was inadequate for diagnosis. All of the group I patients except two had evidence of recurrence during their chemotherapy. None of the patients from group I had more than one surgical resection. The median performance status prior to chemotherapy was 2; only one patient was given an initial performance status of 4.

Ten patients received combination chemotherapy (group II). Seven of these patients were males and three were females, with a median age of 29 years (range, 12–64). Six of these patients had grade III lesions and three had grade IV lesions. One patient had an unbiopsied thalamic glioma. All of these patients except two demonstrated recurrence during their chemotherapy. Two patients had more than one surgical resection. The median performance status at the initiation of chemotherapy was 2; no patients were initially classified as having a performance status of 4.

TABLE 3.—Clinical summary of patients treated with BCNU (group I) or BCNU and VM-26 (group II) for grade III and IV astrocytomas

Patient No.	Age	Sex	Tumor		Prior treatment		Wks from diagnosis to chemotherapy	Chemotherapy*	Initial performance status	Survival (mos)	Remarks
			Grade	Location	Surgery	Radiation therapy (rads)					
1	22	F	III	Frontal	+	6000	32	BCNU + VM-26	0	32†	
2	8	F	—	Thalamus	–	6000	71	BCNU	2	25	BCNU-lung
3	38	F	III	Parietal	+	6000	14	BCNU	2	28†	
4	54	M	IV	Parietal	+	6000	28	BCNU→VM-26	1	14	No response to VM-26
5	48	M	III	Cerebellum	+	6500	92	BCNU + VM-26	3	41	
6	60	M	—	Thalamus	–	6000	20	BCNU + VM-26	3	8	
7	54	M	—	Parietal	+‡	6000	14	BCNU→VM-26	2	11	No response to VM-26
8	52	M	III	Parietal	+	6000	14	BCNU→VM-26	2	8	No response to VM-26
9	30	F	III	Frontal	+	6000	38	BCNU + VM-26	2	24	
10	46	M	IV	Temporal	+§	6000	4	BCNU + VM-26	1	13	
11	49	M	IV	Parietal	+	6100	12	BCNU→VM-26	0	13	Allergic reaction to VM-26
12	62	F	III	Frontal	+	6000	8	BCNU	3	7	
13	64	M	III	Parietal	+	6000	8	BCNU + VM-26	2	4	
14	33	F	III	Cerebellum	+	5600	12	BCNU→VM-26	3	20	No response to VM-26
15	12	M	III	Parietal	+	6000	8	BCNU + VM-26	2	27†	
16	28	M	III	Parietal	+	6000	8	BCNU + VM-26	2	29†	Chemotherapy stopped after 2 yrs
17	13	F	IV	Parietal	+	6000	12	BCNU + VM-26	2	12	
18	22	M	IV	Temporal	+	6000	14	BCNU + VM-26	2	20	
19	17	F	—	Pons	–	6000	14	BCNU	2	10	BCNU-lung
20	20	F	III	Parietal	+	6000	16	BCNU→VM-26	4	46†	No response to VM-26
21	14	F	IV	Thalamus	+	6000	12	BCNU→VM-26	2	18	No response to VM-26

*BCNU + VM-26 = patients received agents initially in combination; BCNU = patients received BCNU alone; BCNU→VM-26 = patients received VM-26 after failing BCNU alone.

†Still alive.

‡Tumor missed at biopsy.

§Had 2 additional resections.

A summary of the entire study population and the results of treatment is shown in table 3. Only five of the 21 patients are alive, two from group I and three from group II. None of the 21 patients demonstrated a 1-unit improvement in performance status. Both of the two group I survivors had grade III lesions and are alive 28 and 46 months after the date of diagnosis. Neither patient had evidence of disease according to CAT brain scanning prior to or after chemotherapy. Three patients with grade III lesions from group II are still alive 27, 29, and 32 months after the date of diagnosis. Two of these patients had no evidence of disease according to CAT brain scanning prior to or after chemotherapy. One patient is alive with recurrent disease. The median survival of group I and II patients was 14 months and 22 months, respectively ($P > 0.05$ by the Wilcoxon rank-sum method). None of the group I patients who received VM-26 following failure with BCNU alone improved. There was no difference between the two groups with respect to the average number of patient-days that they received Decadron. The hematologic toxicity of groups I and II is summarized in table 4. Three patients required rbc transfusions as a result of cumulative BCNU myelotoxicity. Five patients developed significant thrombocytopenia (platelets $< 50{,}000/mm^3$) but no significant hemorrhagic episodes occurred. Neutropenia was the most frequent myelotoxic effect and four patients developed neutrophil counts of $< 1500/mm^3$, but there were no episodes of sepsis or other significant infections. There was no statistical difference in the neutrophil, platelet, or hemoglobin count nadirs between the two groups.

Two patients (Nos. 2 and 19) developed irreversible

TABLE 4.—Hematologic toxicity of groups I and II

	Wbc count (cells/mm³)	Platelet count (cells/mm³)	Hemoglobin level (g/100 ml)
Group I: BCNU alone			
Median nadir	3400	96,000	9.9
Range	900–10,200	30–333,000	5.5–13.4
	(<1500: 2 patients)	(<50,000: 2 patients)	(<8: 2 patients)
Group II: BCNU + VM-26			
Median nadir	2300	81,500	10.6
Range	900–3400	30–150,000	7.7–13.3
	(<1500: 2 patients)	(<50,000: 3 patients)	(<8: 1 patient)

and fatal interstitial pulmonary fibrosis while receiving BCNU. These two patients demonstrated progressive dyspnea without fever and chest roentgenograms revealed a diffuse interstitial pattern. Both patients underwent lung biopsy which demonstrated an interstitial fibrosis with minimal inflammatory cells present. There was no evidence of bacterial or viral infection, fungi, or *Pneumocystis carinii*. These two patients received 960 mg (881 mg/m²) and 2340 mg (903 mg/m²) of BCNU, respectively, in six 2-day cycles over a 6-month period. These doses are similar to cumulative doses of BCNU received by other patients in this study. Our two patients developed lung toxicity over a relatively short period of time in contrast to the only other known case of "BCNU-lung," in which the patient received 3330 mg (2081 mg/m²) over a 3-year period (10). Interestingly, this latter patient had a cerebellar astrocytoma. One patient (No. 11) experienced an idiosyncratic reaction to an initial dose of VM-26, demonstrating hypotension, dyspnea, and bronchospasm. A rechallenge with a smaller dose of VM-26 at a later date produced similar signs and symptoms.

We attempted to determine whether any correlation existed between chemotherapy and periodic examination of the brain by CAT scanning (table 5).

Those patients receiving BCNU alone failed to show any change in tumor area or edema. BCNU combined with VM-26 was ineffective. Based on eight patients, the average weighted value (according to the scale shown in table 5) is +1.25 for tumor area and +1.0 for tumor edema. This means that both the tumor and edema increased slightly during the period of combined chemotherapy.

BCNU therapy followed by VM-26 seems to be no more efficacious than combined or simultaneous therapy. The weighted averages for CAT scan changes based on seven patients are as follows: for those patients receiving BCNU therapy alone the average weighted value is +1.14 for the tumor area and +0.71 for edema. The same seven patients then treated with VM-26 demonstrated an average tumor area value of +1.0 and an average edema value of +0.56. No tumor regression was noted in any patients while they were receiving chemotherapy.

DISCUSSION

Median survival times for patients with high-grade astrocytomas receiving combined modality therapy have ranged from 7 to 15 months. Nitrosourea compounds remain the mainstay of chemotherapy for primary tumors of the central nervous system, largely as a result of their lipid solubility and accessibility to the tumor after systemic administration. Significant improvements in survival have not occurred when chemotherapy is administered after surgery and radiation therapy, although some optimistic reports might suggest improvement in survival when chemotherapy is combined with radiation therapy. In this report, VM-26 was combined with BCNU in an attempt to demonstrate whether such a combination might have an additive influence on survival. Although the median survival of patients in the BCNU and VM-26 group was 22 months compared to 14 months in those patients in the BCNU-alone group, there was no statistical evidence that there was a difference between the two groups. It is acknowledged that our chemotherapy population is a selected group, since one third of the initial population died before the completion of radiation therapy.

Furthermore, none of the patients demonstrated a 1-unit improvement in initial performance status, suggesting that damage incurred as a result of prior tumor destruction, surgery, or both, was largely permanent. Obviously, patients with only slight neurologic impairment or nondominant hemisphere lesions enjoyed a higher quality of life than those patients with severe initial impairment or dominant side lesions. Four of the five patients still alive had no evidence of disease prior to or upon completion of their chemotherapy. We suspect that these patients had their tumor burden reduced substantially after surgery and radiation therapy. The role of chemotherapy in these patients may have been an adjuvant one, or may not have contributed to enhanced survival.

We encountered two deaths from "BCNU-lung" (pulmonary fibrosis). Hematologic toxicity was acceptable,

TABLE 5.—Correlation of chemotherapy and CAT scan findings

Patient No.	Chemotherapy*	Tumor area†	Edema area†	No. of wks of chemotherapy‡	Tumor regression
1	BCNU + VM-26	0	0	76	No
2	BCNU	0	0	32	No
3	BCNU	0	0	40	No
4	BCNU ⟶	+2	+1	12	No
	VM-26	+1	0	8	No
5	BCNU + VM-26	+2	0	68	No
7	BCNU ⟶	0	0	12	No
	VM-26	0	0	4	No
8	BCNU ⟶	+2	0	4	No
	VM-26	+2	–1	8	No
9	BCNU + VM-26	+2	+2	52	No
10	BCNU + VM-26	+2	+2	36	No
11	BCNU ⟶	0	0	32	No
	VM-26	+1	+2		No
12	BCNU§				
13	BCNU + VM-26			4	
14	BCNU ⟶	+2	+2	44	No
	VM-26	+1	+1	28	
15	BCNU + VM-26	0	0	100	No
16	BCNU + VM-26	0	0	108	No
17	BCNU + VM-26	+1	+1	24	No
18	BCNU + VM-26	+1	+1	24	No
19	BCNU	0	0	24	No
20	BCNU ⟶	+1	+1	32	No
	VM-26	0	0	40	No
21	BCNU ⟶	+1	+1	32	No
	VM-26	+2	+2	24	No

*See 1st footnote in table 3.

† 0 = unchanged; +1 = mild enlargement; +2 = marked enlargement; –1 = mild decrease; –2 = marked decrease.

‡ Wks of chemotherapy to point of drug failure, toxicity, or end of study period.

§ Patient failed to return to clinic and no scan was performed to evaluate therapy; patient died.

as were the transient nausea and vomiting associated with drug administration.

We conclude that there is no statistical difference in median survival between BCNU alone and BCNU plus VM-26 as administered to patients in this study with grade III or IV astrocytomas, although a larger series may detect an additive effect with VM-26. Newer methods of early detection as well as newer agents must be developed if real gains are to be made in the management of high-grade astrocytomas. We wonder if the addition of chemotherapy immediately after diagnosis may enhance performance status and survival.

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Analysis and Interpretation of Response Rates for Anticancer Drugs [1]

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SUMMARY

A statistical method for interpreting data on the efficacy of anticancer agents is proposed. Tables were prepared for the retrospective analysis of studies evaluating the activity of anticancer agents against individual tumor types. The number of responding patients and the number of patients entered in the study were used to more objectively and formally determine the future disposition of the drug. Several examples of clinical studies show that use of our tables allows a more satisfactory and reliable classification than is often currently reported.

[Cancer Treat Rep 63:1713–1720, 1979]

A critical review of available data is an important step in planning new clinical trials, setting directions for anticancer treatment research, and evolving new concepts in cancer studies. The decision to undertake further investigation of new anticancer agents often depends on retrospective analyses of response rate data obtained from phase II clinical trials. The method used to interpret such data should identify the activity of a drug in the patient population studied as one of the following:

1. The treatment is not effective.
2. The treatment is effective.
3. The data are inconclusive on the efficacy of the treatment.

Basing the identification solely on the observed response rate can be misleading unless the drug is evaluated in a large number of patients. Confidence-interval procedures are sometimes useful, but quite frequently explicit decision rules concerning the disposition of the drug are a more appropriate and easily interpretable method of evaluation (1–3). For example, if ten responses are observed in 50 patients, then the 95% confidence interval for the true response rate is 9%–31%. Unless the drug is evaluated in a fairly large number of patients, yielding narrow confidence intervals, the confidence-interval procedures lack formal guidelines on deciding whether the drug deserves further evaluation.

Cancer is lethal in nature, and more effective treatments are needed in a majority of tumor types. It therefore is generally agreed that identifying an active drug as inactive is a much more serious mistake than accepting an inactive drug as active. Clinicians are often willing to take as high as a 50% chance of incorrectly identifying a drug as active, but are reluctant to take a $> 5\%$ chance of incorrectly dismissing a drug as inactive. Confidence-interval procedures are not suitable for these asymmetric situations.

The method presented in this paper should be able to account for the disparity in error tolerances and is easy to use with the tables provided, the entries of which are not approximations. More objective assessment of existing data by this method should facilitate a more adequate understanding of the anticancer activity of a drug in a specified disease.

METHOD

Suppose that the available data on a drug show that there were k responses in a sample of n patients. We wish to categorize the drug as effective for this type of patient if the sample supports the hypothesis that the true response rate is at least 100r%. The response rate of 20% (ie, $r = 0.20$) in patients with advanced breast carcinoma previously treated with chemotherapy or in patients with advanced colorectal carcinoma not previ-

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ously treated with chemotherapy is an example of a widely accepted reference response rate. The investigator may classify the drug data into one of three categories:

1. $>$ 100r%: the drug data support at least a 100r% response rate.

2. $<$ 100r%: the drug data support a $<$ 100r% response rate.

3. IC(100r%): the drug data are inconclusive for a 100r% response rate.

Classifying a drug which is $>$ 100r% effective as $<$ 100r% is the false-negativity (FN) error and the tolerable size of its probability P(FN) is denoted by β. Classifying a drug which is $<$ 100r% effective as $>$ 100r% is the false-positivity (FP) error and the tolerable size of its probability P(FP) is denoted by α.

A conventional decision rule satisfying the given tolerances β and α is to decide $>$ 100r% if at least k_1 responses occur in n patients, or to decide $<$ 100r% if at most k_2 responses occur in n patients, or to conclude IC(100r%) otherwise, where the cutoff points k_1 and k_2 are determined so that P(FN) and P(FP) may not exceed β and α respectively. To implement this rule, for each n, r, α, and β the tabulation of (k_1,k_2) is required. Such tabulation of (k_1,k_2) is lengthy unless the maximum tabulated n is a moderate value. Even though it is possible to use a proper approximation method for the above rule beyond a certain n, it is obviously preferable to have a decision rule which can be implemented using short and simple tables without resorting to an approximation unless n is extremely large.

An alternative method of tabulating the tables was sought. For given response rate data (k,n) and tolerable sizes of risk (α,β), the tabulation based on the size of n compared to k was tried, and this required a much shorter table. The reason for producing short tables is that, with a fixed (α,β), (n_1,n_2) varies much faster in k than (k_1,k_2) varies in n. The tables are different from the conventional tables which rely on the size of k compared to n.

Given k responses in n patients, the rule to use the alternative tables is: decide $>$ 100r% if n is $\leq n_1$, decide $<$ 100r% if n is $\geq n_2$, and conclude IC(100r%) otherwise. The n_1 and n_2 can be determined so that

$$P(FN) \leq \sum_{i=0}^{k} \binom{n}{i} r^i (1-r)^{n-i} \leq \sum_{i=0}^{k} \binom{n_2}{i} r^i (1-r)^{n_2-i} \leq \beta \text{ for any } n \geq n_2$$

and

$$P(FP) \leq \sum_{i=k}^{n} \binom{n}{i} r^i (1-r)^{n-i} \leq \sum_{i=k}^{n_1} \binom{n_1}{i} r^i (1-r)^{n_1-i} \leq \alpha \text{ for any } n \leq n_1$$

for given β and α. In particular, since $\beta \leq \alpha < 0.5$ in this paper, n_2 is always $> n_1$.

The advantage of the proposed rule is that the tabulations of (n_1,n_2) for k, α, and β can be covered in a page even though the maximum value of the sample size can be as high as 737 (table 1). It would not be possible to prepare a one-page table like table 1 covering a sample size that large for the conventional rule.

Computations were made for different reference rates of effectiveness ranging from 10% to 90% considering a P(FN) of 0.05 and P(FP)s of 0.05 and 0.5 (tables 1 and 2). For example, suppose that the data show seven responses in 22 patients, and that the treatment is to be evaluated relative to a reference rate of 20%. According to table 1, when a treatment yields seven responses, its degree of activity is ≥ 0.20 if the number of patients in the sample is ≤ 17, whereas its degree of activity is < 0.20 if the sample size is ≥ 63. In this particular example, with seven responses in 22 patients the activity of the treatment is classified as inconclusive [IC(20%)]. If table 2 is used, where the P(FP) is 10 times greater than in table 1, the treatment is accepted for further trial, but in this case there is a $<$ 50% chance that the treatment is actually ineffective. If the same data are evaluated with respect to the 15% reference response rate, table 1 shows that the drug is $>$ 15% effective and the P(FP) is at most 0.05.

The table can also be used for reference rates $>$ 50%. Suppose there are 32 responses to a drug in 38 patients and it must be determined if the response rate is at least 70%. This is equivalent to saying that there are six nonresponders in 38 patients and that the nonresponse rate of interest is 30%. For six responses and a 30% response rate, from table 1, the nonresponse rate of 30% is rejected since 38 is greater than 37. This implies that the drug can be classified as 70% effective.

Table 3 is added for the readers willing to accept $\beta = 0.1$ and/or $\alpha = 0.1,0.2$. Example data in this paper are all analyzed only by tables 1 and 2. An explanation of how to use each table appears in the footnotes to the tables.

RESULTS

The usefulness of this method will be illustrated with examples from published reviews on the single-agent activity of anticancer agents. Using table 1, a proposed ranking of activity has been determined for high-dose methotrexate in several tumor types (table 4), for bleomycin in head and neck carcinoma (table 5), and for phase II single agents in bilharzial bladder cancer (table 6). The anticancer activity is classified relative to the reference response rate of 20%. Classifications according to other pertinent percentages have also been determined. The chance that each classification is erroneous is less than one in 20.

The data on activity of Adriamycin in various tumor types were taken from the review by Wasserman et al (2). No explanations were made available to support the validity of the mode of drug assessment used in the original clinical investigations. The analyses of the data using

TABLE 1.—Maximum and minimum sample size necessary to accept or reject a new treatment at a chosen rate of effectiveness [P(FP) and P(FN) each not > 0.05]*

No. of responses	Rate of effectiveness										No. of responses
	> 10%	< 10%	> 15%	< 15%	> 20%	< 20%	> 30%	< 30%	> 50%	< 50%	
0		29		19		14		9		5	0
1		46		30		22		14		8	1
2	3	61	2	40	2	30		19		11	2
3	8	76	6	50	4	37	3	24		13	3
4	14	89	10	59	7	44	5	28		16	4
5	20	103	14	68	10	50	7	33	5	18	5
6	27	116	18	76	14	57	10	37	6	21	6
7	34	129	23	85	17	63	12	41	8	23	7
8	41	142	28	93	21	69	14	45	9	26	8
9	48	154	33	102	25	76	17	49	11	28	9
10	56	167	38	110	29	82	20	53	13	30	10
11	63	179	43	118	32	88	22	57	14	33	11
12	71	191	48	126	36	94	25	61	16	35	12
13	79	203	53	134	40	100	28	65	18	37	13
14	86	215	58	142	44	106	30	69	19	40	14
15	94	227	64	150	48	112	33	73	21	42	15
16	102	239	69	158	52	118	36	77	23	44	16
17	110	251	74	166	56	124	39	81	24	47	17
18	119	263	80	174	61	129	41	85	26	49	18
19	127	275	85	182	65	135	44	89	28	51	19
20	135	286	91	190	69	141	47	93	30	53	20
21	143	298	96	197	73	147	50	96	31	56	21
22	152	310	102	205	77	153	53	100	33	58	22
23	160	321	108	213	81	158	55	104	35	60	23
24	168	333	113	220	86	164	58	108	37	62	24
25	177	345	119	228	90	170	61	112	38	65	25
26	185	356	125	236	94	176	64	115	40	67	26
27	194	368	130	243	99	181	67	119	42	69	27
28	202	379	136	251	103	187	70	123	44	71	28
29	211	391	142	259	107	193	73	127	45	74	29
30	219	402	147	266	111	198	76	130	47	76	30
31	228	413	153	274	116	204	79	134	49	78	31
32	236	425	159	281	120	210	81	138	51	80	32
33	245	436	165	289	124	215	84	142	53	82	33
34	254	447	170	296	129	221	87	145	54	85	34
35	262	459	176	304	133	227	90	149	56	87	35
36	271	470	182	311	138	232	93	153	58	89	36
37	280	481	188	319	142	238	96	156	60	91	37
38	289	493	194	326	146	243	99	160	62	93	38
39	297	504	200	334	151	249	102	164	63	96	39
40	306	515	205	341	155	255	105	168	65	98	40
41	315	526	211	349	160	260	108	171	67	100	41
42	324	537	217	356	164	266	111	175	69	102	42
43	332	549	223	364	168	271	114	179	71	104	43
44	341	560	229	371	173	277	117	182	72	106	44
45	350	571	235	379	177	282	120	186	74	109	45
46	359	582	241	386	182	288	123	190	76	111	46
47	368	593	247	393	186	293	126	193	78	113	47
48	377	604	253	401	191	299	129	197	80	115	48
49	385	615	259	408	195	304	132	201	82	117	49
50	394	627	265	416	200	310	135	204	83	119	50
51	403	638	270	423	204	315	138	208	85	122	51
52	412	649	276	430	209	321	141	212	87	124	52
53	421	660	282	438	213	326	144	215	89	126	53
54	430	671	288	445	218	332	147	219	91	128	54
55	439	682	294	452	222	337	150	223	93	130	55
56	448	693	300	460	227	343	153	226	94	132	56
57	457	704	306	467	231	348	156	230	96	135	57
58	466	715	312	474	236	354	159	233	98	137	58
59	475	726	318	482	240	359	162	237	100	139	59
60	484	737	324	489	245	365	165	241	102	141	60

**How to use the table:* Enter the No. of responses in the table. Choose a true effectiveness rate of interest. If the No. of patients treated is less than or equal to the No. in the left column, the treatment is at least as effective as the chosen rate. If it is greater than or equal to the No. in the right column, the treatment is inferior to the reference rate chosen. If the No. is in between, no conclusion can be drawn at the rate chosen.

TABLE 2.—Maximum and minimum sample size necessary to accept or reject a new treatment at a chosen rate of effectiveness [P(FP) not > 0.5 and P(FN) not > 0.05]*

No. of responses	Rate of effectiveness										No. of responses
	> 10%	< 10%	> 15%	< 15%	> 20%	< 20%	> 30%	< 30%	> 50%	< 50%	
0		29		19		14		9		5	0
1	6	46	4	30	3	22	1	14	1	8	1
2	16	61	10	40	8	30	5	19	3	11	2
3	26	76	17	50	13	37	8	24	5	13	3
4	36	89	24	59	18	44	11	28	7	16	4
5	46	103	30	68	23	50	15	33	8	18	5
6	56	116	37	76	28	57	18	37	10	21	6
7	66	129	44	85	33	63	21	41	12	23	7
8	76	142	50	93	38	69	25	45	14	26	8
9	86	154	57	102	43	76	28	49	16	28	9
10	96	167	64	110	48	82	31	53	18	30	10
11	106	179	70	118	53	88	35	57	20	33	11
12	116	191	77	126	58	94	38	61	22	35	12
13	126	203	84	134	63	100	41	65	25	37	13
14	136	215	90	142	68	106	45	69	27	40	14
15	146	227	97	150	73	112	48	73	29	42	15
16	156	239	104	158	78	118	51	77	30	44	16
17	166	251	110	166	83	124	55	81	32	47	17
18	176	263	117	174	88	129	58	85	35	49	18
19	186	275	124	182	93	135	61	89	37	51	19
20	196	286	130	190	98	141	65	93	39	53	20
21	206	298	137	197	103	147	68	96	40	56	21
22	216	310	144	205	108	153	71	100	42	58	22
23	226	321	150	213	113	158	75	104	44	60	23
24	236	333	157	220	118	164	78	108	47	62	24
25	246	345	164	228	123	170	81	112	49	65	25
26	256	356	170	236	128	176	85	115	51	67	26
27	266	368	177	243	133	181	88	119	52	69	27
28	276	379	184	251	138	187	91	123	54	71	28
29	286	391	190	259	143	193	95	127	56	74	29
30	296	402	197	266	148	198	98	130	59	76	30
31	306	413	204	274	153	204	101	134	61	78	31
32	316	425	210	281	158	210	105	138	62	80	32
33	326	436	217	289	163	215	108	142	64	82	33
34	336	447	224	296	168	221	111	145	66	85	34
35	346	459	230	304	173	227	115	149	68	87	35
36	356	470	237	311	178	232	118	153	71	89	36
37	366	481	244	319	183	238	121	156	72	91	37
38	376	493	250	326	188	243	125	160	74	93	38
39	386	504	257	334	193	249	128	164	76	96	39
40	396	515	264	341	198	255	131	168	78	98	40
41	406	526	270	349	203	260	135	171	80	100	41
42	416	537	277	356	208	266	138	175	82	102	42
43	426	549	284	364	213	271	141	179	84	104	43
44	436	560	290	371	218	277	145	182	86	106	44
45	446	571	297	379	223	282	148	186	88	109	45
46	456	582	304	386	228	288	151	190	90	111	46
47	466	593	310	393	233	293	155	193	92	113	47
48	476	604	317	401	238	299	158	197	94	115	48
49	486	615	324	408	243	304	161	201	96	117	49
50	496	627	330	416	248	310	165	204	98	119	50
51	506	638	337	423	253	315	168	208	100	122	51
52	516	649	344	430	258	321	171	212	102	124	52
53	526	660	350	438	263	326	175	215	104	126	53
54	536	671	357	445	268	332	178	219	106	128	54
55	546	682	364	452	273	337	181	223	108	130	55
56	556	693	370	460	278	343	185	226	110	132	56
57	566	704	377	467	282	348	188	230	112	135	57
58	576	715	384	474	287	354	191	233	114	137	58
59	586	726	390	482	292	359	195	237	116	139	59
60	596	737	397	489	297	365	198	241	118	141	60

**See* table 1 for directions on how to use the table.

TABLE 3.—Maximum and minimum sample size necessary to accept or reject a new treatment at a chosen rate of effectiveness*

No. of responses	Rate of effectiveness										No. of responses
	> 10%	< 10%	> 15%	< 15%	> 20%	< 20%	> 30%	< 30%	> 50%	< 50%	
0		22		15		11		7		4	0
1	(2)	38	(1)	25	(1)	18		12		7	1
2	5 (8)	52	3 (5)	34	(4)	25	2 (2)	16		9	2
3	11 (15)	66	7 (10)	43	6 (7)	32	4 (5)	21	(3)	12	3
4	18 (23)	78	12 (15)	52	9 (11)	38	6 (8)	25	4 (5)	14	4
5	25 (31)	91	17 (21)	60	13 (15)	45	9 (10)	29	5 (6)	17	5
6	32 (39)	104	22 (26)	68	16 (20)	51	11 (13)	33	7 (8)	19	6
7	40 (47)	116	27 (32)	77	20 (24)	57	14 (16)	37	9 (10)	21	7
8	47 (56)	128	32 (37)	85	24 (28)	63	16 (19)	41	10 (12)	24	8
9	55 (65)	140	37 (43)	93	28 (32)	69	19 (22)	45	12 (13)	26	9
10	63 (73)	152	42 (49)	100	32 (37)	75	22 (25)	49	14 (15)	28	10
11	71 (82)	164	48 (55)	108	36 (41)	81	25 (28)	53	15 (17)	31	11
12	79 (91)	175	53 (61)	116	40 (46)	86	27 (31)	57	17 (19)	33	12
13	88 (100)	187	59 (67)	124	45 (50)	92	30 (34)	60	19 (21)	35	13
14	96 (109)	199	64 (73)	132	49 (55)	98	33 (37)	64	21 (22)	37	14
15	104 (118)	210	70 (79)	139	53 (59)	104	36 (40)	68	22 (24)	39	15
16	113 (126)	222	76 (85)	147	57 (64)	109	39 (43)	72	24 (26)	42	16
17	121 (136)	233	81 (91)	154	62 (68)	115	42 (46)	76	26 (28)	44	17
18	130 (145)	245	87 (97)	162	66 (73)	121	45 (49)	79	28 (30)	46	18
19	138 (154)	256	93 (103)	170	70 (77)	126	47 (52)	83	29 (32)	48	19
20	147 (163)	267	99 (109)	177	75 (82)	132	50 (55)	87	31 (34)	51	20
21	156 (172)	279	104 (115)	185	79 (86)	138	53 (58)	91	33 (35)	53	21
22	164 (181)	290	110 (121)	192	83 (91)	143	56 (61)	94	35 (37)	55	22
23	173 (190)	301	116 (127)	200	88 (96)	149	59 (64)	98	37 (39)	57	23
24	182 (199)	312	122 (133)	207	92 (100)	154	62 (67)	102	39 (41)	59	24
25	191 (208)	324	128 (139)	215	96 (105)	160	65 (70)	105	40 (43)	62	25
26	199 (218)	335	134 (146)	222	101 (110)	166	68 (74)	109	42 (45)	64	26
27	208 (227)	346	140 (152)	229	105 (114)	171	71 (77)	113	44 (47)	66	27
28	217 (236)	357	146 (158)	237	110 (119)	177	74 (80)	116	46 (49)	68	28
29	226 (245)	368	151 (164)	244	114 (123)	182	77 (83)	120	48 (50)	70	29
30	235 (255)	379	157 (170)	252	119 (128)	188	80 (86)	124	49 (52)	72	30
31	244 (264)	390	163 (176)	259	123 (133)	193	83 (89)	127	51 (54)	75	31
32	253 (273)	402	169 (183)	266	128 (137)	199	86 (92)	131	53 (56)	77	32
33	262 (282)	413	175 (189)	274	132 (142)	204	89 (95)	135	55 (58)	79	33
34	270 (292)	424	181 (195)	281	137 (147)	210	92 (98)	138	57 (60)	81	34
35	279 (301)	435	187 (201)	288	141 (151)	215	95 (102)	142	59 (62)	83	35
36	288 (310)	446	193 (208)	296	146 (156)	221	98 (105)	146	60 (64)	85	36
37	297 (320)	457	199 (214)	303	150 (161)	226	101 (108)	149	62 (66)	87	37
38	306 (329)	468	205 (220)	310	155 (165)	232	104 (111)	153	64 (68)	90	38
39	315 (338)	479	211 (226)	318	159 (170)	237	107 (114)	156	66 (69)	92	39
40	324 (348)	490	217 (232)	325	164 (175)	243	110 (117)	160	68 (71)	94	40
41	333 (357)	501	223 (239)	332	168 (180)	248	113 (120)	164	70 (73)	96	41
42	343 (367)	511	229 (245)	339	173 (184)	253	117 (124)	167	72 (75)	98	42
43	352 (376)	522	236 (251)	347	178 (189)	259	120 (127)	171	73 (77)	100	43
44	361 (385)	533	242 (258)	354	182 (194)	264	123 (130	174	75 (79)	102	44
45	370 (395)	544	248 (264)	361	187 (198)	270	126 (133)	178	77 (81)	105	45
46	379 (404)	555	254 (270)	368	191 (203)	275	129 (136)	182	79 (83)	107	46
47	388 (414)	566	260 (276)	376	196 (208)	281	132 (139)	185	81 (85)	109	47
48	397 (423)	577	266 (283)	383	200 (213)	286	135 (143)	189	83 (87)	111	48
49	406 (432)	588	272 (289)	390	205 (217)	291	138 (146)	192	85 (89)	113	49
50	415 (442)	599	278 (295)	397	210 (222)	297	141 (149)	196	87 (90)	115	50
51	424 (451)	609	284 (302)	405	214 (227)	302	144 (152)	200	88 (92)	117	51
52	434 (461)	620	290 (308)	412	219 (232)	308	147 (155)	203	90 (94)	119	52
53	443 (470)	631	296 (314)	419	223 (236)	313	150 (158)	207	92 (96)	122	53
54	452 (480)	642	303 (321)	426	228 (241)	318	153 (162)	210	94 (98)	124	54
55	461 (489)	653	309 (327)	433	233 (246)	324	157 (165)	214	96 (100)	126	55
56	470 (499)	664	315 (333)	441	237 (250)	329	160 (168)	218	98 (102)	128	56
57	479 (508)	674	321 (339)	448	242 (255)	334	163 (171)	221	100 (104)	130	57
58	489 (517)	685	327 (346)	455	246 (260)	340	166 (174)	225	102 (106)	132	58
59	498 (527)	696	333 (352)	462	251 (265)	345	169 (177)	228	103 (108)	134	59
60	507 (536)	707	339 (358)	469	256 (269)	351	172 (181)	232	105 (110)	136	60

**How to use the table:* Enter the No. of responses in the table. Choose a true effectiveness rate of interest. If the No. of patients treated is less than or equal to the No. in the left column, the treatment is at least as effective as the chosen rate; the P(FP) is at most 0.1. If the No. of patients is less than or equal to the No. in parentheses, the treatment is at least as effective as the chosen rate; the P(FP) is at most 0.2. If the No. of patients is greater than or equal to the No. in the right column, the treatment is inferior to the reference rate chosen. Otherwise, no conclusion can be drawn at the rate chosen.

TABLE 4.—Activity of high-dose methotrexate (ref 4)

Tumor type	No. of responses/ No. of patients treated	Observed response rate (%)	Proposed ranking— At 20%	Proposed ranking— At other %
Sarcoma	17/56	30	> 20%	IC (30%)
Osteogenic	11/26	42	> 20%	IC (30%)
Ewing's	1/4	25	IC (20%)	
Rhabdomyosarcoma	1/5	20	IC (20%)	
Miscellaneous	4/21	19	IC (20%)	
Lymphoma	27/38	71	> 20%	> 50%
Non-Hodgkin's	23/30	77	> 20%	> 50%
Unspecified	4/8	50	IC (20%)	> 15%
Head and neck	26/37	70	> 20%	> 50%
Brain	12/25	48	> 20%	> 30%
Glioma	7/17	41	> 20%	IC (30%)
Medulloblastoma	4/7	57	> 20%	IC (30%)
Ependymoma	1/1	100	IC (20%)	
Breast	6/23	26	IC (20%)	> 10%
Melanoma	8/22	36	IC (20%)	> 15%
Lung	3/20	15	IC (20%)	
Small cell	2/8	25	IC (20%)	
Non-small cell	1/12	8	IC (20%)	
Pancreas	5/18	28	IC (20%)	> 10%
Cervix	3/8	38	IC (20%)	> 10%
Anus	2/4	50	IC (20%)	
Acute myelocytic leukemia	1/2	50	IC (20%)	

TABLE 5.—Activity of bleomycin in head and neck cancer (ref 5)

Dose (mg/m^2)	Schedule	Route	No. of responses/ No. of patients treated	Observed response rate (%)	Proposed ranking— At 20%	Proposed ranking— At other %
10, 15, 30 15, 30	Twice weekly Daily × 5–8	iv	28/62	45	> 20%	> 30%
20	Twice weekly	iv or im	24/53	45	> 20%	> 30%
10	Twice weekly	iv vs im	12/64	19	IC (20%)	> 10%
10–20 20	Daily × 10–38 Twice weekly	iv or im	9/54	17	IC (20%)	
9.25	Daily to toxicity	iv	6/46	13	IC (20%)	IC (15%)
10	Twice weekly	iv vs im	5/81	6	< 20%	< 15%

TABLE 6.—Activity of single agents in bilharzial bladder cancer

Agent	No. of responses/ No. of patients treated	Ranking— At 20%	Ranking— At other %	Ref No.
Methotrexate	2/42	< 20%	< 15%	6
Hexamethylmelamine	10/26	> 20%		7
VM-26	1/24	< 20%		7
Bleomycin	0/21	< 20%	< 15%	8

*The No. of patients entered in the trial.

TABLE 7.—Activity of Adriamycin (ref 2)

Tumor type	No. of responses/ No. of patients treated	Observed response rate (%)	Reported assessment*	Proposed ranking— At 20%	Proposed ranking— At other %
Ovary	18/48	38	+ +	> 20%	IC (30%)
Breast	67/193	35	+ +	> 20%	
Bladder	27/97	28	+ +	> 20%	IC (30%)
Sarcoma	88/351	25	+ +	> 20%	
Lung	53/314	17	+ +	< 20%	> 10%
Cervix	9/28	32	+	IC (20%)	> 15%
Stomach	7/27	26	+	IC (20%)	> 10%
Testicle	12/60	20	+	IC (20%)	> 10%
Head and neck	13/67	19	+	IC (20%)	> 10%
Colon	8/92	9	–	< 20%	
Kidney	4/56	7	–	< 20%	
Melanoma	0/19	0	–	< 20%	< 15%
Uterus	2/6	33	NE	IC (20%)	
Prostate	2/9	22	NE	IC (20%)	

*NE = not evaluated; – = adequate evaluation, drug inactive; + = inadequate evaluation, evidence of drug activity but not clearly established; + + = adequate evaluation, drug definitely active.

table 1 are presented in table 7. The proposed classification indicates that Adriamycin is at least 10% active in lung, stomach, testicle, and head and neck cancer patients, whereas it is at least 15% active in cervical cancer. Among these five tumor types, Wasserman et al (2) classified Adriamycin as active only in lung cancer, although, based on their cumulative results, Adriamycin is < 20% effective in lung cancer and is IC(20%) in the others. On the other hand, if Adriamycin is considered active in ovarian tumors because the observed response rate is 38%, this classification can be misleading. According to this method, Adriamycin is > 20% active but is IC(30%) in this disease.

Bellet et al (1) ranked and classified the antitumor activity in malignant melanoma according to "the probability of true response rate greater than a specified rate" of 10% (table 8). This probability is equivalent to the size of a P(FN) (β) and the method does not take into account the P(FP).

The results of Bellet have been re-analyzed according to the method described in this paper (table 8); only two drugs are acceptable at the minimum response rate of 10% for α and β set at 0.05. If the α level is increased to 0.5, 12 more drugs are accepted.

DISCUSSION

The statistical method presented in this paper allows a more adequate assessment of the efficacy of anticancer treatment than the apparent response rate does, which often fails to reflect the reliability of the interpretation rendered by the sample size. An assumption underlying the computation is that the probability of responding to treatment is identical for each patient evaluated. This emphasizes the impropriety of pooling several reports without a careful review of the prognostic characteristics and the differing factors (eg, time, measurement criteria, etc) of each study. In addition, whether or not a drug is

TABLE 8.—Activity of single agents in malignant melanoma (ref 1)

Agent	No. of responses/ No. of patients treated	Observed response rate (%)	Probability of true response rate > 10%	Reported assessment*	Proposed ranking at 10% ($\alpha = \beta = 0.05$)	Proposed ranking at 10% ($\alpha = 0.5$, $\beta = 0.05$)
BCNU	24/140	17.1	99.6	1	> 10%	
Methyl-CCNU	24/158	15.2	98.6	1	> 10%	
ThioTEPA	5/24	20.8	97.2	1	IC (10%)	> 10%
TMCA	13/90	14.4	93.7	2	IC (10%)	> 10%
TEPA	3/16	18.8	93.2	2	IC (10%)	> 10%
O-PAM	4/24	16.7	91.5	2	IC (10%)	> 10%
Dibromodulcitol	3/18	16.7	90.2	2	IC (10%)	> 10%
Mitomycin C	9/65	13.8	88.9	2	IC (10%)	> 10%
Methotrexate	2/13	15.4	86.6	2	IC (10%)	> 10%
Cyclophosphamide	4/32	12.5	78.9	3	IC (10%)	> 10%
Vinblastine	7/58	12.1	78.0	3	IC (10%)	> 10%
Vincristine	3/26	11.5	74.1	3	IC (10%)	> 10%
TIC mustard	3/26	11.5	74.1	3	IC (10%)	> 10%
CCNU	17/153	11.1	73.1	3	IC (10%)	> 10%
Cytosine arabinoside	3/27	11.1	71.8	3	IC (10%)	
Streptozotocin	2/19	10.5	70.5	3	IC (10%)	
Chlorambucil	2/22	9.1	62.0	3	IC (10%)	
6-Mercaptopurine	2/29	6.9	43.5	4	IC (10%)	
5-Fluorouracil	1/20	5.0	39.2	4	IC (10%)	
Hydroxyurea	7/86	8.1	36.2	4	IC (10%)	
Hexamethylmelamine	2/42	4.8	19.5	4	IC (10%)	
ICRF-159	0/20	0.0	17.6	4	IC (10%)	
Pregnane	11/155	7.1	14.0	4	IC (10%)	
Bleomycin	1/40	2.5	8.1	4	IC (10%)	
Mechlorethamine	0/25	0.0	7.2	4	IC (10%)	
Actinomycin D	0/30	0.0	4.2	4	< 10%	
Adriamycin	0/32	0.0	3.4	4	< 10%	

*1 = clinically useful (rejecting the drug as < 10% effective will result in at least a 95% chance of false negativity); 2 = potentially useful (rejecting the drug as < 10% effective will result in an 80%–95% chance of false negativity); 3 = probably not useful (rejecting the drug as < 10% effective will result in a 50%–80% chance of false negativity); 4 = inactive (rejecting the drug as < 10% effective will result in at most a 50% chance of false negativity).

useful for a particular patient population can be properly determined only by prospective studies. This information cannot be obtained by extrapolating results achieved in other populations.

Retrospective reviews of the literature and existing data are indispensable processes in designing prospective trials and formulating new cancer treatments. The method presented in this report should help define the efficacy of a drug in a specified patient population, and makes possible quick, easy, and objective assessments of literature data. It highlights the importance of the number of observations in lending validity to a particular reference rate, and it precludes invalid rankings of activity based on percentages from relatively preliminary data as often reported in the literature.

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Two-Stage Plans for Patient Accrual in Phase II Cancer Clinical Trials [1]

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SUMMARY

This paper considers two-stage patient-accrual plans for phase II trials of anticancer drugs. The purpose is to identify the therapeutic level of drugs as either above or below a reference response rate. Using such a plan, decisions concerning the future disposition of drugs can be made more formally and objectively. The tables used to implement the plan are prepared for a 20% reference response rate, due to its wide acceptance in the design of phase II clinical trials.

[Cancer Treat Rep 63:1721–1726, 1979]

SECTION 1. INTRODUCTION

The objective of phase II trials of anticancer drugs is to identify the therapeutic efficacy of new treatments. The end-point in most phase II trials is response rate, response usually being defined as objective tumor regression (eg, ≥ 50% of the cross-sectional tumor area). Such studies are expected to yield a definitive answer on whether a new treatment regimen is likely to be effective in a defined patient population and is therefore accepted or discarded for subsequent testing. The design and methods of phase II clinical trials have been the subjects of several papers by statisticians as well as clinicians (1–4). [4]

A widely used design is the following: enter 14 patients and if at least one response is obtained, then enter sufficiently more patients so that the response rate is estimated with the desired precision (1). Informal designs are often used in phase II clinical trial protocols. The total number of patients accessible for the study is predetermined (usually ranging from 25 to 40), and then patients are admitted to the study in two stages, the size of the first group being somewhere between 14 and 20 and the second stage accrual following if some antitumor activity is observed in the first group. Most such designs entail the estimation of the response rate with a relatively wide confidence interval. For example, if ten responses are observed in 50 patients, the 95% confidence interval for the true response rate is 9%–31%. Unless the drug is evaluated in a fairly large number of patients yielding a narrow confidence interval, these estimation procedures generally lack any formal guidelines useful in deciding whether the drug deserves further evaluation.

There are three factors to be considered in designing patient-entry plans in phase II clinical trials.

1. The number of patients available for evaluating new anticancer treatments is limited. One of the constraints in planning cancer clinical trials is that the sample size is a consequence of the availability of eligible patients rather than statistical rationale. When the sample size for a phase II clinical trial is predetermined, procedures for estimating the response rate have a more or less predetermined precision (roughly $1/(2\sqrt{n})$ [n = number of patients] is the standard deviation) and sometimes are less than instrumental in determining the next mode of evaluation for a drug, especially when n is not large.
2. Because of the lethal nature of untreated cancer, it is generally agreed that dismissing an active drug is a much more serious mistake than accepting for further study a drug with inadequate antitumor activity.

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3. In evaluating a new drug there is concern that it may be toxic without discernible anticancer activity. This concern discourages subjecting a more than minimally required number of patients to a drug whose therapeutic potency is yet to be evaluated in man.

Three kinds of patient-accrual plans are possible for phase II trials. Using a fixed-sampling plan, a predetermined number of patients are studied and then the anticancer activity of the drug is evaluated. For example, one of the guidelines proposed by Staquet and Sylvester (2) is to evaluate 14 patients and if there are no responses dismiss the drug as inactive. This plan entails a high probability of false positivity. Increasing the number of patients decreases the size of the false positivity probability (2), but a large number of patients is required to achieve this effect. The third factor cited above generally discourages clinicians to subject, in a single stage, a large number of patients to a new drug.

A fully sequential approach is possible for phase II trials. The sequentially arriving patients are evaluated one at a time and it is decided after each evaluation whether or not to enter another patient. The sequential plan is satisfactory from the viewpoint of the consideration mentioned above, because it is known to require, on the average, a smaller number of patients than the fixed-sampling plan or the stage-wise plan discussed below (5). One difficulty in applying the sequential plan to chronic-disease studies is that the time to response may be longer than the patient-arrival interval. Another difficulty is that there is a delay between the time of evaluation and the time of reporting, especially in multi-institutional studies, which are often necessary for phase II studies.

The third approach is a stage-wise plan. At each stage a fixed number of patients are evaluated and if the data enable the investigators to make a definitive inference concerning the anticancer activity of the drug, the patient acerual terminates; otherwise, the next predetermined number of patients are admitted to the trial. Although there are other stage-wise designs, two-stage designs are frequently used because they are simple and avoid the difficulties associated with the fixed-sampling or sequential plans.

This paper considers the two-stage patient-accrual plans for phase II trials that will identify the therapeutic level of the drug as above or below a reference response rate, thus enabling investigators to decide more formally and objectively whether or not to discard the drug. The plans that we present in this paper are adopted from Armitage (6) and are modified to meet the constraint that the size of the clinical trial is predetermined by nonstatistical considerations such as the availability of patients. The tables used to implement the plans are prepared for a 20% reference response rate, due to its wide acceptance in the design of many phase II clinical trials. The tables can also be prepared for other response rates of interest to adapt to the particular situation under study. A nonmyelosuppressive drug might be acceptable at a lower response rate; similarly, in diseases such as pancreatic cancer, melanoma, or lung cancer a 10%–15% response rate might still be worth noting and may warrant additional testing. For convenience of presentation we use 20% as the reference response rate throughout this paper.

SECTION 2. REQUIREMENTS FOR PATIENT-ACCRUAL PLAN

The phase II clinical trial may be considered a drug screening process. Drugs showing promising therapeutic activity will be accepted for additional clinical evaluation, while those not likely to be effective are discarded from further consideration. The number of patients in a phase II study is often determined by the availability of eligible patients, but the design requires that the probability of rejecting an effective drug (denoted by β) be within a given tolerance.

Keeping the β probability low will result in a high probability of false positivity (α), ie, accepting an ineffective drug. Table 1 displays the β and α probabilities when deciding whether the true response rate is $> 20\%$ or $< 20\%$ based upon an observed number of responses with a predetermined number of patients evaluated. For example, deciding that the response rate is $> 20\%$ for ten responses in 40 patients results in a 26.8% chance of accepting an ineffective drug. Another decision rule would be to say, for instance, that the true response rate is $< 20\%$ for three or fewer responses among 40 patients and $> 20\%$ for 13 or more responses, while withholding a decision for intermediate results. In this case both β and α probabilities are < 0.05, but the chance of inconclusive findings in a clinical trial with 40 patients is intolerably high (0.928). Thus, when the number of available patients is limited, the design should balance the risks of erroneous decision (β and α) and the chance of reaching no conclusion.

In order to obtain designs tolerable for all three risks, we consider those that satisfy the following constraints:

(*a*) The probability of rejecting a drug (β) that is $> 20\%$ effective must not exceed 0.05 regardless of the number of patients (n) evaluated.

(*b*) The probability of accepting a drug (α) that is $< 20\%$ effective must not exceed 0.5 for $n \leq 35$, 0.4 for $n \geq 40$ but ≤ 45, and 0.3 for $n \geq 50$.

(*c*) The average probability of withholding a decision for a drug $< 10\%$ effective and for a drug $> 30\%$ effective must not exceed 0.4 for $n \leq 45$ and 0.3 for $n \geq 50$.

The first two probabilities are β and α probabilities for the 20% reference response rate, and the third quantity will be denoted by PA(IC) (IC = inconclusive). If the

TABLE 1.—False-negative probability of < 20% conclusion and false-positive probability of > 20% conclusion in the case of fixed-sampling patient-accrual plan

No. of responses	n = 14		n = 25		n = 40	
	β	α	β	α	β	α
0	0.044	1.000	0.004	1.000	0.000	1.000
1	0.198	0.956	0.027	0.996	0.001	1.000
2	0.448	0.802	0.098	0.973	0.008	0.999
3	0.700	0.552	0.234	0.902	0.028	0.992
4	0.870	0.302	0.421	0.766	0.076	0.972
5	0.956	0.130	0.617	0.579	0.161	0.924
6	0.988	0.044	0.780	0.383	0.286	0.839
7	1.000	0.012	0.891	0.220	0.437	0.714
8		0.002	0.953	0.109	0.593	0.563
9		0.000	0.983	0.047	0.732	0.407
10			0.994	0.017	0.839	0.268
11			0.998	0.006	0.912	0.161
12			1.000	0.002	0.957	0.088
13				0.000	0.981	0.043

maximum tolerance for one probability is lowered without compensating either of the other two probabilities, there may be no two-stage plan or fixed-sampling plan which simultaneously satisfies all probabilistic requirements.

The considered operating characteristics of the patient-accrual plans are:

1. The probability of rejecting a ≥ 20% effective drug for further clinical trials, β.
2. The probability of accepting a < 20% effective drug for further clinical trials, α.
3. The probability of accepting a ≥ 30% effective drug, P(TP) (TP = true positive).
4. The average of two probabilities of inconclusive data, one when the drug is at least 30% effective and the other when the drug is at most 10% effective, PA(IC).
5. The probability of rejecting a ≤ 10% effective drug, P(TN) (TN = true negative).
6. The sample size of the first stage, n_1.
7. The average of two probabilities of early termination (terminating the trial after the first stage), one when the drug is at least 30% effective and the other when the drug is at most 10% effective, PA(ET).
8. The average number of patients to be admitted to the trial, n_{av}.

The 0.05 β probability is well-accepted in phase II cancer clinical trials. The α probability is set higher to achieve a small value for the β probability (consistent with point 2 in Section 1) and to reduce the number of inconclusive clinical trials. The tolerance level of α varies with n in this paper but is never > 0.5. In this way, effective drugs are identified with a high probability [ie, high P(TP)], and a drug with even a hint of anticancer activity may not be rejected [ie, relatively low P(TN)] but may instead be found inconclusive in its activity. The smaller the PA(IC), the higher the chance of establishing therapeutic efficacy of a drug at the end of the trial. A small n_1 is desirable (*see* point 3 in Section 1) so that no more than the minimal necessary number of patients will be submitted to a trial of a new drug with unknown activity. A large PA(ET) value implies that the drug with strong activity will be identified as active and the inactive drug will be rejected with high probability after the first stage, an obvious advantage. Suppose that there are k clinical trials using the same plan. Since some of the trials may end after the first stage, the average number of patients may be smaller than n. The n_{av} reflects this. The average sample size is determined by

$$n_{av} = n_1 + [n - n_1] \times [1 - PA(ET)].$$

SECTION 3. OPTIMAL TWO-STAGE PLAN

Suppose that the maximum number of patients available for a phase II study is specified in advance as n. The two-stage design is as follows:

1. Admit n_1 patients during the first stage. If there are at least a_1 responders, stop the trial and decide that the drug is effective with a 20% response rate (> 20%).
2. If there are r_1 or fewer responders, stop the trial and decide that the drug is ineffective with a 20% response rate (< 20%).
3. If the number of responders is between r_1 and a_1 (exclusive), continue to the second stage of patient accrual and enter additional patients into the trial (the total number of patients is n).
4. If there are at least a_2 responders in all n patients, decide that the drug is effective (> 20%).
5. If there are r_2 or fewer responders in all n patients, decide that the drug is ineffective (< 20%).
6. If the number of responders is between r_2 and a_2 (ex-

TABLE 2.—Optimal 2-stage patient-accrual plan

n	n_1	a_1	r_1	a_2	r_2
25	15	4	0	6	1
30	19	6	0	7	2
35	15	5	0	8	2
40	16	5	0	10	3
45	18	6	0	11	4
50	25	7	0	13	5
60	19	6	0	15	6

clusive) in all n patients, withhold the decision on the efficacy of the drug.

The set of constants (n_1, a_1, r_1, a_2, and r_2) for the above design is presented for n = 25, 30, 35, 40, 45, 50, and 60 in table 2. The chosen set of constants is the one with the smallest possible n_1 and/or the smallest expected sample size among all the sets of constants satisfying the probabilistic requirements stated in (*a*), (*b*), and (*c*) in Section 2. The appendix presents a detailed description of how the constants are obtained.

[*Example:* Suppose a nonrandomized phase II clinical trial is planned. Considering the past ability to accrue patients meeting the eligibility criteria, the size of the trial is set at 40 patients. The optimal two-stage plan is determined from table 2. *a*. Enter 16 patients first. *b*. If no response occurs, discard the drug as < 20% effective. *c*. If five or more responses are observed, accept the drug as > 20% effective. *d*. If one to four responses are observed, enter 24 more patients. *e*. If three or fewer responses are observed among 40 patients, discard the drug as < 20% effective. *f*. If ten or more responses are observed, accept the drug as > 20% effective. *g*. If two to nine responses are observed, withhold the decision on the efficacy of the drug.

As can be seen from table 3, this plan guarantees $\beta < 0.049$, $\alpha < 0.34$, and PA(IC) < 0.34.]

Other characteristics of this design are as follows (*see* table 3): (*a*) If the drug is > 30% effective, the chance of accepting the drug is at least 84% [P(TP) > 0.84]. (*b*) If the drug is < 10% effective, the chance of rejecting the drug is at least 46% [P(TN) > 0.46]. (*c*) The average probability of terminating the trial after the first stage when the true unknown response rate is at least 30% or at most 10% is > 38%.

Whether or not to discard a drug whose therapeutic efficacy is not firmly established after a well-designed clinical trial with an acceptable number of patients depends on the availability of effective drugs for the patient population studied and the overall prognostic characteristics of the patients evaluated. It also can be determined based on the retrospective analysis at a different response rate as suggested in the previous paper in this issue (7).

Table 3 shows that the optimal two-stage plan and the fixed-sampling plan are quite comparable with regard to β, α, PA(IC), P(TP), and P(TN). The advantage of the optimal two-stage plan clearly lies in the possibility of early termination of the trial.

SECTION 4. TWO-STAGE PLAN WITH FIXED FIRST-STAGE SIZE

One of the popular patient-accrual plans in phase II clinical trials is as follows: enter 14 patients and admit more patients only if there is at least one responder. This design is based on the concept that rejecting a drug showing no response in 14 consecutive patients risks a < 5% chance of dismissing a drug > 20% effective (1). A two-stage plan based on this criterion is presented in this section. Its initial stage size is set at 15 patients, because with 14 patients in the first stage we could not find, for the sample sizes considered in this paper, a two-stage design having a β probability < 0.05 for a drug > 20% effective. Again, the total sample size (n) is assumed to be prespecified according to the ability to accrue eligible patients. The two-stage plan with 15 patients in the first stage is the same as the plan described in Section 3 except that n_1 is replaced by "15."

The set of constants (a_1, r_1, a_2, and r_2) to implement the above plan is presented for n = 25, 30, 35, 40, 45, and 50 in table 4. The constants for some values of n do not satisfy the probabilistic requirements set forth in Section 2. In such cases, constants are chosen which satisfy the requirements most closely. When several sets of constants are competing, the set with the smallest average sample size is selected. The probability of rejecting an effective drug (β) is always kept at < 0.05. Operating characteristics are summarized in table 3. The two-stage plan with the initial stage size is inferior to the other plans except in PA(ET) and n_{av}.

SECTION 5. DISCUSSION

The two-stage plans presented in this paper have been developed to meet the constraints laid down by the three factors cited in Section 1. According to the plans, a drug with little or no activity may be rejected and a drug with definite activity may be identified after the first stage with a small number of patients. When an intermediate result occurs after the first stage, the second-stage patient entry follows. The design characteristics of the plans are prepared to meet the second consideration, with a small β (0.05), a tolerable α, and infrequent inconclusive results.

Even though the sample size is a consequence of the ability to accrue eligible patients, a generally desirable sample size depends on the drug status and the responsiveness of the tumor type under study. For nonresponsive tumor types (advanced colorectal cancer, advanced

TABLE 3.—Operating characteristics of patient-accrual plans

Plan	n	n_1	a_1	r_1	a_2	r_2	β	α	PA(IC)	PA(ET)	n_{av}	P(TP)	P(TN)
Fixed-sampling	25	25	6	1			0.027	0.38	0.47	0.00	25	0.81	0.27
	30	30	7	2			0.044	0.39	0.36	0.00	30	0.84	0.41
	35	35	8	2			0.019	0.40	0.40	0 00	35	0.87	0.31
	40	40	10	3			0.028	0.27	0.38	0 00	40	0.80	0.42
	45	45	11	4			0.038	0.28	0.32	0 00	45	0.84	0.53
	50	50	13	5			0.048	0.19	0.30	0.00	50	0.78	0.62
	60	60	15	6			0.031	0.21	0.27	0.00	60	0.84	0.61
Optimal 2-stage	25	15	4	0	6	1	0.049	0.46	0.38	0.48	21	0.84	0.33
	30	19	6	0	7	2	0.050	0.40	0.35	0.34	27	0.84	0.42
	35	15	5	0	8	2	0.047	0.42	0.36	0.34	28	0.87	0.37
	40	16	5	0	10	3	0.049	0.34	0.34	0.38	31	0.84	0.46
	45	18	6	0	11	4	0.050	0.31	0.31	0.31	37	0.85	0.55
	50	25	7	0	13	5	0.050	0.29	0.27	0.37	41	0.83	0.62
	60	19	6	0	15	6	0.042	0.28	0.25	0.34	47	0.86	0.62
2-stage with 1st stage size fixed at 15	15	15	4	0			0.035	0.35	0.51		15	0.70	0.21
	25	15	4	0	6	1	0.049	0.46	0.38	0.48	21	0.84	0.33
	30	15	4	0	7	1	0.040	0.49	0.39	0.48	23	0.88	0.28
	35	15	4	0	8	2	0.047	0.42	0.36	0.34	28	0.87	0.37
	40	15	4	0	11	2	0.040	0.39	0.40	0.48	28	0.82	0.32
	45	15	4	0	13	3	0.044	0.37	0.38	0.48	31	0,80	0.40
	50	15	4	0	14	4	0.049	0.38	0.31	0.48	34	0.83	0.49

TABLE 4.—2-stage patient-accrual plan with the initial stage size fixed at 15

n	n_1	a_1	r_1	a_2	r_2
15	15	4	0		
25	15	4	0	6	1
30	15	4	0	7	1
35	15	5	0	8	2
40	15	4	0	11	2
45	15	4	0	13	3
50	15	4	0	14	4

malignant melanoma, or tumors failing the standard treatment), the α probability and P(TN) probability are not so compelling as the β probability, and the number of early phase II drugs is fairly large compared to the number of eligible patients. The recommended design is ($n = 25$, $n_1 = 15$) or ($n = 35$, $n_1 = 15$), depending on the ability to accrue patients. Drugs demonstrating promising activity in some tumors are usually evaluated in patients with responsive (as well as nonresponsive) tumor types (eg, lymphomas, small cell lung carcinoma, ovarian carcinoma). The recommended design here is ($n = 40$, $n_1 = 16$), ($n = 45$, $n_1 = 18$), ($n = 50$, $n_1 = 25$), or ($n = 60$, $n_1 = 19$), depending on the patient-accrual ability, so that the α, P(TP), and P(TN) probabilities may be reasonable and more definitive conclusions concerning the drug may be drawn.

The two-stage plans require, on the average, fewer patients than comparable fixed-sampling plans when the true response rate deviates from 20% in either direction. In particular, in a "master" protocol which randomizes among various phase II treatment arms, substantial savings in patient resources result from employing the two-stage plans.

The assumption underlying the phase II trial designs is that the probability of responding to treatment is identical for each patient included in the trial. This emphasizes the necessity for a well-defined patient population and the impropriety of extrapolating the study results to other patient populations.

We assumed that the sample size n would be predetermined by the patient-accrual consideration. But even if the sample size were a consequence of statistical considerations, the two-stage design presented is equally applicable. For example, a popular n is 25, and the statistical justification is that the estimated response rate has a roughly 10% standard deviation. Suppose there were six responders among 25 patients. The observed response rate is 24% and the 95% confidence interval is 9.4%–45.1% (8). The decision approach presented in this paper suggests that the drug is effective (table 2) for the patient population. Thus, even if the design consideration is based on estimating the response rate with a desired precision, the two-stage plan presented is applicable and complements the confidence-interval procedure in deciding the next mode of evaluation of the drug.

Finally, we note that n_1 in table 2 and a_1 in table 4 are not monotone in n. This is caused by the discrete nature of the count data.

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APPENDIX

The following briefly describes how table 2 is generated. Call (n, n_1, a_1, r_1, a_2, and r_2) the design point. For a given n, candidate design points satisfying the probabilistic requirements set forth in Section 2 are determined. Candidate design points are then further reduced by screening out ones with large n_1 and n_{av} values. The final choice of the design point is made among the few remaining candidates based on the overall quality of each candidate. For example, in the case of $n = 25$, there are only seven candidate design points satisfying the requirements of $\beta < 0.05$, $\alpha < 0.5$, and PA(IC) < 0.4. They are:

n	n_1	a_1	r_1	a_2	r_2	β	α	PA(IC)	n_{av}
25	15	4	0	6	1	0.049	0.46	0.38	21
25	16	4	0	6	1	0.043	0.49	0.37	21
25	17	4	0	7	1	0.039	0.47	0.39	21
25	18	4	0	9	1	0.035	0.50	0.38	22
25	18	4	0	10	1	0.035	0.50	0.39	22
25	22	5	1	6	1	0.038	0.48	0.37	24
25	22	5	1	7	1	0.048	0.46	0.38	24

From the seven candidates, we select (25, 15, 4, 0, 6, and 1) as the design point, because it has the smallest n_1 and n_{av} values. Not all cases are as simple as $n = 25$. P(TP) and P(TN) are added to table 3 after design points are selected. Finally, we note that since the probability of accepting a drug < 10% effective and the probability of rejecting a drug > 30% effective are both very small, PA(IC) is roughly

$$1 - [P(TP) + P(TN)]/2$$

where P(TP) represents the probability of accepting a drug > 30% effective and P(TN) represents the probability of rejecting a drug < 10% effective.

Detailed mathematic properties and methods of locating design points are presented in an unpublished manuscript "Two-Stage Designs for Drug Screening When the Sample Size is Prespecified," a copy of which may be obtained by writing to Dr. Young Jack Lee.

A Mathematic Model for Relating the Drug Sensitivity of Tumors to Their Spontaneous Mutation Rate [1]

J. H. Goldie* and A. J. Coldman [2,3]

SUMMARY

A mathematic model has been developed relating the drug sensitivity of a tumor to its own spontaneous mutation rate towards phenotypic drug resistance. The proportion as well as the absolute numbers of resistant cells will increase with time and the fraction of resistant cells within tumor colonies of the same size will vary depending on whether mutation occurs as an early or late event. Analysis of the model indicates that the probability of the appearance of a resistant phenotype increases with the mutation rate. Furthermore, for any population of tumors with a non-zero mutation rate the likelihood of there being at least one resistant cell will go from a condition of low to high probability over a very short interval in the tumor's biologic history.

[Cancer Treat Rep 63:1727–1733, 1979]

Current programs of adjuvant chemotherapy are based on the assumption that tumor micrometastases can be cured by courses of drug therapy that would not be curative for clinically advanced disease. There is a considerable body of both experimental and clinical data that support the belief that small numbers of tumor cells can be eradicated readily by chemotherapy, and that this probability of cure diminishes and then is lost as the tumor burden increases (1–3).

Many factors are known to contribute to the refractoriness to treatment of advanced tumors. With increasing size of tumor burden the growth kinetics of the tumor will become progressively less favorable for chemotherapeutic effect. There will be metabolic and immunologic effects on the host and an increased likelihood of residence by tumor in pharmacologic sanctuaries.

Most mathematic models of tumor growth are primarily concerned with such kinetic factors as size of growth fraction and mean generation time of component cells (4–6). There is recognition that the development of resistant mutants within a tumor constitutes a significant factor in determining response to drug treatment, but generally this phenomenon is assigned a subordinate degree of importance as compared with the kinetic processes indicated above. In antimicrobial chemotherapy, the presence of drug-resistant strains is usually the crucial factor which determines the success of antibiotic treatment. To the extent that analogous processes may occur within tumor cell populations, one would predict that the presence or absence of drug-resistant lines within the tumor would be a decisive factor in determining whether or not a neoplastic cell line could be eradicated by chemotherapy.

In this paper we will describe a model for the probability of occurrence of drug-resistant cells within tumor colonies of various sizes based on somatic cell mutation theory. The model predicts that there will be variation in the size of the resistant fraction within tumor colonies of the same size for any given value of the mutation rate, and furthermore that there will be a sudden decline in the expectation for cure as the cell number increases. It is suggested that this has certain implications for the timing of adjuvant chemotherapy.

THEORY

There is substantial evidence suggesting that drug-resistant mutations in mammalian tumor cells arise by a

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process analogous to that seen in microbial populations (7–11). That is to say, drug-resistant phenotypes arise spontaneously and with a definite frequency, independent of the selecting agent. It has been argued that the data do not permit distinction between genetic and epigenetic processes (12); however, evidence has been presented recently that is consistent with the hypothesis that true genetic mutations resulting in drug resistance do occur in mammalian cells (13–15). It is also true, however, that epigenetic processes may still be operative in some circumstances. In this paper, it will be assumed that stable genetic alterations do arise in tumor cells resulting in phenotypic change in drug sensitivity.

To avoid unnecessarily complicating this model a few simplifying assumptions have been made. We have assumed that all cells within the tumor have stem cell capacity, that we have available to us only a single-drug treatment modality, and that a resistant phenotype is totally resistant to the drug (ie, cannot be killed by any pharmacologically achievable drug concentration).

Of course, in real situations multiple agents are available to the therapist and tumor cells can be expected to show varying degrees of resistance, from reduced sensitivity to total refractoriness. Confining attention to one agent simplifies the problem without removing the generality of the solution, while the assumption of a totally resistant phenotype is an essential hypothesis of this model.

An expression relating the mean size of the resistant population to the total tumor size may be derived as follows. Let μ be the mean size of the resistant population (R) and let N be the size of the tumor. Then if α is the mutation rate per cell generation, it may be shown (*see* appendix I) that μ will satisfy the following differential equation

$$\frac{d\mu}{dN} = \frac{\mu}{N} + \alpha\left\{1 - \frac{\mu}{N}\right\}$$

which yields

$$\mu = \{1 - N^{-\alpha}\}\, N \qquad [1]$$

after requiring $\mu = 0$, when $N = 1$. From this it may be seen that the mean fraction of resistant cells (F) increases with increasing size of the tumor; ie,

$$F = \frac{\mu}{N} = \frac{\{1 - N^{-\alpha}\}N}{N} = \{1 - N^{-\alpha}\}.$$

This is another way of saying that the phenotypic diversity of the population being considered will increase with size, assuming that mutations from S (sensitive phenotype) ⟶ R (resistant phenotype) are stable and have a constant frequency. This relationship between the size of a subpopulation of resistant phenotypes and the total population being considered was first derived by Luria and Delbruck (11) in their classic paper on mutations to resistance in bacterial populations.

At this point R has been assumed to be a state from which there is no back mutation. If we assume that back mutation would occur at the same rate, then it may be shown (*see* appendix I) that the expected number of resistant cells is given by

$$\mu = \frac{1}{2}\{1 - N^{-2\alpha}\}N \qquad [2]$$

which for $N < 10^{10}$ and $\alpha < 0.001$ is not significantly numerically different from $\{1 - N^{-\alpha}\}N$ and thus, unless the rate of back mutation is much greater than that of forward mutation, its effect will be negligible and will be ignored hereafter.

Figure 1 plots the logarithm of the expected number of the resistant population (μ) versus $\log_{10}N$ for various values of the mutation rate (α). The values of $\alpha(10^{-3}, 10^{-4}, 10^{-5}, 10^{-6})$ are consistent with what has been estimated for mammalian cell populations (7). It may be seen from the illustration that the rate of increase of μ exceeds that of the total population and will approach it in the absence of back mutation as may be inferred from equation [1].

The probabilistic nature of this process implies that there will be a distribution of possible values of R for a given tumor size. Mutations early in the growth of the tumor will lead to large values for the resistant subpopulation. The small but finite probability for such an event causes the distribution of R to be highly skewed, as is illustrated in figure 2, where a computer simulation of the process is presented. This shows that the mean of the distribution is far removed from its mode and thus

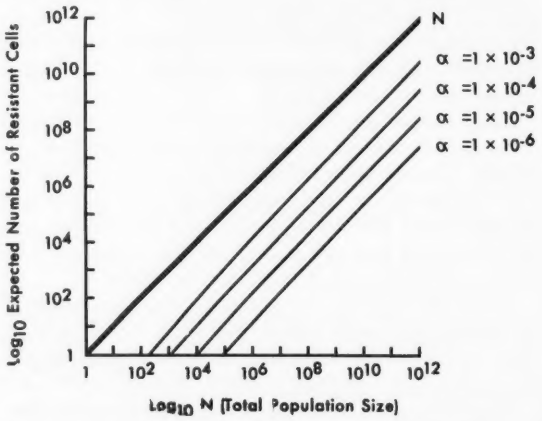

FIGURE 1.—Plot of the log of the expected mutant population vs the log of the total tumor population for 4 values of the mutation rate (α). The straight line indicating the growth in total population is included as a reference. The lines for the resistant subpopulation are slightly curved and approach the total population line, illustrating that the mutant population increases at a greater rate than the total tumor population. This relationship will hold no matter what growth function is assumed for the tumor as a whole.

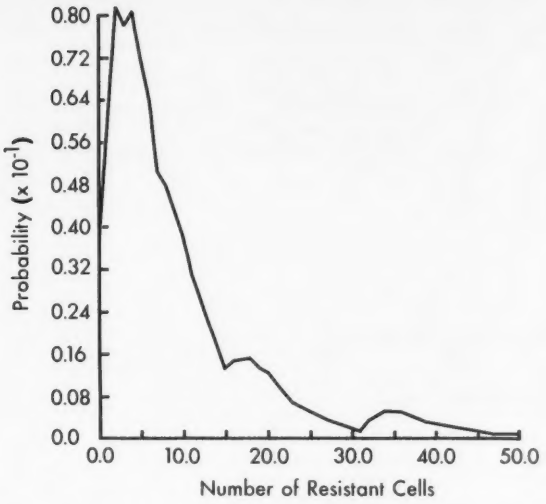

FIGURE 2.—Plot of a computer simulation of the frequency distribution of the No. of resistant cells that would be found in a collection of tumors of size 3.2×10^4 cells and having a mutation rate (α) = 1×10^{-4}. The simulation was obtained by splitting the growth of the tumor into discrete intervals and considering the No. of mutations in each interval to follow a Poisson law with appropriate means. This method led to powers of 2 being favored and artificially created the "bumps" shown close to powers of 2. Nevertheless, the figure reveals the true "skewed" nature of the process.

the mean is not a complete indicator of the behavior of such a process.

The point at which the curve in figure 2 cuts the y axis represents the probability of finding 0 resistant cells in a tumor which is given precisely by the formula (*see* appendix II)

$$P = \exp\{-\alpha(N - 1)\} \qquad [3]$$

where α and N are as before. This function is plotted in figure 3 and is seen to decrease with increasing N for a given α, as would be expected. The resistant state could be composed of any cells with reduced susceptibility to a particular chemotherapy. If we assume that its members are completely resistant to that treatment, and if the treatment given is sufficient to destroy the "susceptible cells," then P is the probability of cure for a tumor of size N. As tumor size increases the probability of resistant clones increases, and thus for reasons quite independent of growth kinetics we would expect increasing resistance to treatment with increased tumor size. Although P is never 0, when the product of α N is > 4 the presence of a resistant cell is almost certain and therefore from this one would predict a large number of resistant populations in clinically advanced tumors, ie, where $N \sim 10^{11}$ and $\alpha = 10^{-5}$ and therefore $\alpha N \sim 10^6$. Of course, since the proportion of sensitive cells will almost always be much greater than the resistant fraction, the tumor can be expected to exhibit temporary responsiveness to therapy until the size of the resistant fraction approximates that of the sensitive fraction.

The development of a resistant clone by any group of tumors is a random event and the time for it to occur will be a function of the growth curve of the tumor and the mutation rate (α). A quantity which is useful as a measure of the variability of the process is the time taken for a tumor population to go from a high probability to a low probability of 0 resistant clones. This quantity is seen to be dependent upon the growth curve of the tumor; however, the required log increase in growth (ie, the actual range of growth over which the change occurs) is independent of the growth curve. This may be calculated as follows:

$$P(N) = \exp\{-\alpha(N - 1)\}.$$

Therefore

$$\log_e P(N) = -\alpha(N - 1).$$

Therefore for N_1, $N_2 >> 1$

$$\log_{10}\left\{\frac{\log_e P(N_2)}{\log_e P(N_1)}\right\} = \log_{10}\left(\frac{N_2}{N_1}\right) = \text{log increase in growth}$$

which is seen to be independent of the mutation rate. For example, the log increase in growth required to transfer from a 0.95 probability to a 0.05 probability of 0 resistant clones is

$$\text{log increase in growth} = \log_{10}\left\{\frac{\log_{10} P(N_2)}{\log_{10} P(N_1)}\right\} = 1.77.$$

This indicates that at certain times in the growth of a tumor population, there is a period in which its susceptibility to chemotherapy changes rapidly (fig 3).

This has implications for the circumstances under which adjuvant chemotherapy will be used, ie, where one is attempting to eliminate the small residual burden of tumor cells that are left in the host after the primary growth has been removed. During a particular time when the tumor population increases by 1.77 logs (5.9 doublings) the chances of cure will go from a high to a low level of probability. The higher the mutation rate the earlier in the growth of the tumor this transition will occur. Since this interval constitutes a relatively small proportion of the tumor's total growth history, short delays in institution of therapy during this critical transition time will seriously compromise the effectiveness of the therapy. This situation is similar, in certain respects, to the phenomenon observed in the treatment of the transplanted L1210 leukemia, where the curability of the neoplasm is quickly lost as the tumor burden approximates 10^6 (2).

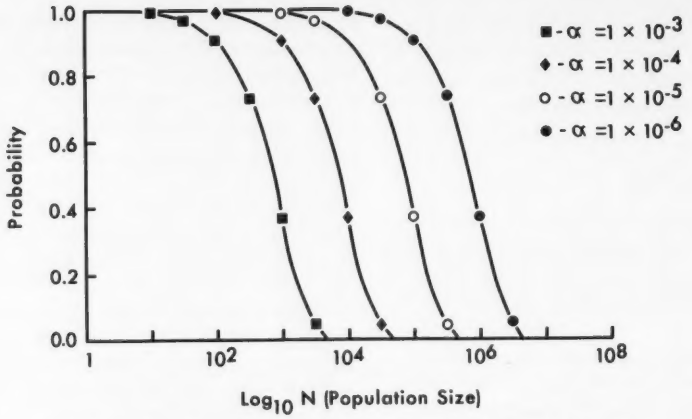

FIGURE 3.—Plot of equation [3] which relates the probability of the existence of no resistant phenotypes to the mutation rate (α) and tumor size (N). The almost identical profile of each curve reflects the independence of the time required for a class of tumors to proceed from the state when only 5% have resistant phenotypes to that when 95% have such phenotypes.

CONCLUSION

In this model we have suggested that the expectation of cure of a malignant neoplasm is a function of (*a*) the spontaneous mutation rate towards resistance, an intrinsic property of the tumor, and (*b*) the size the tumor has reached from the initial transformation to the time when therapeutic intervention begins. Even without the additional effects due to growth kinetics, drug pharmacokinetics, and host defense factors, this minimal model provides a basis for the direction in which adjuvant chemotherapy trials are proceeding. That is to say, tumors early in their growth history can be expected, on stochastic grounds alone, to have a much better likelihood of cure than tumors that have reached an advanced stage.

Although there is no known way of altering α, the spontaneous mutation rate (except that some modalities such as radiation therapy and alkylating agent therapy may actually increase it), the use of combination chemotherapy where both agents are effective and independent in their mode of action will have the effect of reducing the value of α for the tumor. The analogy of combined drug treatment for mycobacterial infection is pertinent here (16).

The therapist does have some control over when treatment is started. The data presented in this paper indicate the importance of the time factor with respect to the initiation of treatment. At a critical point in the development of a tumor when the value of P, that is to say the expectation for cure, begins to fall quickly, delay of even a couple of generation times will greatly reduce the likelihood of cure. A case could be made for initiating adjunctive chemotherapy simultaneously with the attack on the primary tumor or even, circumstances permitting, before primary treatment is begun.

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APPENDIX I

Considering N to be an approximately continuous variable, μ (the expected number of mutants) may be expanded about the point N using Taylor's theorem

$$\mu(N + \Delta N) = \mu + \frac{d\mu}{dN}\Delta N + 0(\Delta N^2). \qquad [i]$$

If the total tumor population increases by ΔN, then there will be an expected $\frac{\mu}{N}\Delta N$ growth in the resistant number, an increase of $\alpha(1 - \frac{\mu}{N})\Delta N$ due to new mutations of S→R, and a decrease of $\beta\frac{\mu}{N}\Delta N$ (where β is the back mutation rate) mutations of R→S. Therefore, the expected number of mutants is

$$\mu(N + \Delta N) = \mu + \frac{\mu}{N}\Delta N + \alpha(1 - \frac{\mu}{N})\Delta N - \beta\frac{\mu}{N}\Delta N + 0(\Delta N^2). \qquad [ii]$$

Equating [i] and [ii], dividing by ΔN, and taking limit $\Delta N \to 0$ yields

$$\frac{d\mu}{dN} = \frac{\mu}{N}(1 - \beta) + \alpha(1 - \frac{\mu}{N}).$$

Using the condition $\mu = 0$ when $N = 1$ yields

$$\mu = \frac{\alpha}{\alpha + \beta}\{1 - N^{-\alpha-\beta}\}N.$$

If there is no back mutation, $\beta = 0$,

$$\mu = \{1 - N^{-\alpha}\}N \qquad [1]$$

and if the back mutation rate = the forward mutation rate, ie, $\alpha = \beta$

$$\mu = \frac{1}{2}\{1 - N^{-2\alpha}\}N. \qquad [2]$$

APPENDIX II

If P is the probability of 0 resistant colonies in a tumor of size N, then we may expand P around N using Taylor's theorem, assuming N continuous

$$P(N + \Delta N) = P + \frac{dP}{dN}\Delta N + 0(\Delta N^2). \qquad [i]$$

The probability that a mutation will occur during a total population increase (ΔN) is approximately distributed as a Poisson process and therefore the probability of no mutations in this period is

$$\exp(-\alpha\Delta N).$$

Using the law of conditional probability (Bayes' theorem)

$$\begin{aligned} P(N + \Delta N) &= P\exp(-\alpha\Delta N) \\ &= P - \alpha P\Delta N + 0(\Delta N^2). \end{aligned} \qquad [ii]$$

Equating [i] and [ii], dividing by ΔN, and taking limit $\Delta N \rightarrow 0$ yields

$$\frac{dP}{dN} = -\alpha P.$$

Using the condition $P = 1$ when $N = 1$ yields

$$P = \exp\{-\alpha(N-1)\}.$$

cis-Dichlorodiammineplatinum(II), Methotrexate, Bleomycin, and Vincristine in Head and Neck Cancer: A Pilot Study[1,2]

Gerald W. King, John J. Halpin, Roy E. Smith, Frank Batley, and David E. Schuller[3,*]

SUMMARY

Thirty-nine patients with advanced epidermoid carcinoma of the head and neck were treated with a combination of *cis*-dichlorodiammineplatinum(II), methotrexate, bleomycin, and vincristine. Twenty-nine patients were evaluable for response and 39 were evaluable for toxicity. With this regimen toxicity was acceptable and the following rates were observed in a total of 139 treatment courses: 100% (nausea and vomiting), 3% (decreased creatinine clearance), 4% (thrombocytopenia), 5% (leukopenia), and 2% (pulmonary fibrosis). There was one death due to sepsis during a period of chemotherapy-induced leukopenia. Although the patients treated with this regimen had advanced disease and had been treated aggressively previously, an overall response rate of 24% was observed, with three patients (10%) having a complete response. Median duration of response was 7+ months. These results indicate that this intensive combination chemotherapy has a sufficiently favorable risk/benefit ratio to allow its evaluation in randomized clinical trials in patients with head and neck cancer.

[Cancer Treat Rep 63:1735–1738, 1979]

Epidermoid carcinomas of the head and neck are notoriously resistant to chemotherapy, especially when this modality is introduced after radiation and/or extensive surgery (1). Single agents are reported to produce response rates in the range of 20%–50%, but complete responses are rare and the duration of response is usually only 2–3 months. Unfortunately, combinations of effective single agents have not been shown to be superior to the individual agent, especially in terms of duration of remission and survival (1).

This grim outlook for patients with advanced head and neck cancer suggests that even more vigorous treatment is indicated. The purpose of this study was to evaluate an extremely potent chemotherapy combination in patients with far-advanced epidermoid carcinomas of the head and neck with respect to toxicity of the regimen as well as response prior to its use in randomized clinical trials. The drugs selected for this combination are among the most effective single agents for advanced disease: methotrexate, *cis*-dichlorodiammineplatinum(II) (*cis*-platin), bleomycin, and vincristine (1–3).

MATERIALS AND METHODS

Thirty-nine patients with advanced head and neck cancer were entered in this study. There were 25 males and nine females with a mean age of 57.8 years. Mean smoking exposure in the 29 patients known to smoke was 49.2 pack years and 44% were moderate to heavy drinkers. All patients had histologically confirmed epidermoid carcinoma. Primary site of disease and extent of metastases at the time of entry in the study are shown in table 1. Previous treatment is presented in table 2. Patients were considered evaluable if they completed at least one full course of chemotherapy. All patients were evaluated for toxicity but only patients receiving one full course of chemotherapy were evaluated for response. All patients had not received chemotherapy for at least 1 month prior to entry and all were clinically judged to have a life ex-

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TABLE 1.—Primary tumor site and extent of involvement

	No. of patients	%
Primary tumor site		
Tongue	6	15
Pharynx	5	13
Nasopharynx	4	10
Hypopharynx	4	10
Larynx	4	10
Tonsil	3	8
Lip	2	5
Buccogingival	2	5
Miscellaneous, other sites	9	23
Extent of disease on entry		
Primary tumor site	37	95
Metastases		
Lymph nodes	19	49
Pulmonary	12	31
Bone	10	26
Subcutaneous	2	5
Liver	1	3

TABLE 2.—Previous therapy (39 patients)

	No. of patients	%
Prior radiation therapy	23	59
Extensive prior surgery	22	56
Prior chemotherapy		
Cyclophosphamide, Adriamycin, vincristine, methotrexate	1	3
Cyclophosphamide, methotrexate, 5-fluorouracil	1	3
Methotrexate	2	5
Methotrexate, bleomycin	2	5
Methotrexate, bleomycin, L-asparaginase	1	3
No prior therapy	7	18

pectancy of at least 12 weeks. Response to treatment was defined as complete response (CR) if all measurable disease disappeared; partial response (PR) if measurable disease decreased $\geq$ 50% in the product of the two maximal diameters; and no response (NR) if disease regression was < 50% or disease progression was noted.

Chemotherapy toxicity was monitored with two weekly physical examinations, weekly cbcs and platelet counts, and by obtaining the following assays monthly prior to each treatment: BUN, creatinine, 24-hour creatinine clearance, pulmonary function tests, chest roentgenograms, audiograms, alkaline phosphatase, and SGOT.

Treatment consisted of *cis*-platin (50 mg/m^2 by iv push on Day 1 of each course), vincristine (2.0 mg by iv push on Days 1 and 14 of each course), methotrexate (40 mg/m^2 by iv push on Days 1 and 14), and bleomycin (15 units/m^2 im on Days 1 and 14). This regimen was repeated every 28 days. Prior to treatment, patients were hydrated at least overnight with 2 liters of 5% dextrose in 0.45 NaCl + 20 mEq of KCl, and immediately before treatment, each patient received 12.5 g of mannitol by iv bolus. After *cis*-platin, each patient received a 6-hour infusion of 2 liters of NaCl + 20 mEq of KCl and 50 g of mannitol. The *cis*-platin dose was adjusted on the basis of creatinine clearance (table 3).

TABLE 3.—*cis*-Platin dose adjusted on the basis of creatinine clearance

Creatinine clearance (ml/min)	*cis*-Platin dose
75	Decrease by 25%
50	Decrease by 50%
40	Decrease by 60%
30	Decrease by 70%
< 30	Stop treatment

The dose of methotrexate was adjusted according to the degree of myelosuppression and mucositis. If the wbc and platelet count nadirs were > 3500 and 150,000/mm^3 respectively, the dose of methotrexate was increased by 25%. If the wbc and platelet count nadirs were $\leq$ 2500 and 75,000/mm^3, or if severe mucositis developed, the dose was decreased by 25%.

RESULTS

As previously mentioned, this study was designed to evaluate the toxicity of this intensive regimen prior to its inclusion in randomized clinical trials. Table 4 shows the toxic effects observed in 39 patients who received a total of 139 courses of chemotherapy. The death thought to be due to chemotherapy toxicity occurred in an elderly patient who died of sepsis with a wbc count of 400/mm^3. Treatment delays due to cytopenias occurred only seven times (5%) and delays due to patient refusal occurred in only two courses (1%), even though 100% of patients had severe nausea and vomiting with each course. There were no treatment delays due to decreased creatinine clearance but the dose of *cis*-platin had to be decreased in three instances (2%) because of impaired creatinine clearance. This relative tolerance of *cis*-platin with our hydration scheme was also demonstrated in two patients who were accidentally given increased doses of *cis*-platin (60 mg/m^2) in the face of creatinine clearances of 35 and 38 ml/minute. There were no problems associated with these "overdoses" and, indeed, in one of these patients the creatinine clearance improved to 60 ml/minute after the *cis*-platin dose. One patient developed a mild high-frequency hearing deficit that did not require cessation

TABLE 4.—Toxic effects of 139 chemotherapy courses

Toxicity	Severity	No. of courses with toxicity (%)
Thrombocytopenia (cells/mm^3)	< 150,000	9 (6)
	< 100,000	5 (4)
Leukopenia (cells/mm^3)	< 2500	11 (8)
	< 2000	7 (5)
Decreased creatinine clearance (ml/min)	≤ 50	4 (3)
Mucositis	Mild	2 (1)
	Moderate	2 (1)
Pulmonary (fibrosis, diffusion, impairment)	Mild	3 (2)
Nausea, vomiting	Moderate	139 (100)
Hypotension	Mild	5 (4)
Ototoxicity	Mild	1 (< 1)
No. of deaths due to toxicity		1 (< 1) (leukopenia)

of treatment. Vincristine was discontinued in seven patients due to neurotoxicity and bleomycin was discontinued in five patients due to hypotension or pulmonary toxicity.

In terms of response to this regimen, table 5 shows the response rates of all patients entered in the study. Twenty-nine patients were evaluable for response. Ten patients were nonevaluable because they (*a*) died before completion of one course, (*b*) were lost to followup, or (*c*) died from toxicity. There were three CRs, four PRs, and 22 NRs in this group of heavily pretreated patients. The patients with CR had primary diagnoses of buccogingival, tonsillar, and tongue epidermoid carcinoma. Two of these three patients had metastatic disease in addition to primary site recurrence and had received radiation therapy and radical surgery prior to entry in the study. All four partial responders had metastatic disease at the time of entry and two had previously received both radical surgery and radiation therapy. One patient had received radiation therapy alone and one had not been treated previously. Primary sites included pyriform fossa, pharynx, hard palate, and nasopharynx. None of the responders had received previous chemotherapy.

CONCLUSIONS

The need for more aggressive chemotherapy for head and neck cancer is obvious when the grim statistics associated with prognosis are evaluated (1). Previous studies (involving small numbers of patients) indicate that the overall response rate for *cis*-platin in combination is from 12% to 50%, and that the toxicity is acceptable (4–6). The combination evaluated in this study contains potent chemotherapeutic agents which are singly effective against this group of tumors but which appear to have significant overlapping toxic effects. Both *cis*-platin and methotrexate are known to be nephrotoxic (3,7–9) and methotrexate and bleomycin are known pulmonary toxins (10–12). Myelosuppression from methotrexate might also be aggravated by *cis*-platin (3,9,13). Thus, it was surprising that these toxic manifestations were actually quite uncommon in our patients, especially considering that most patients had far-advanced disease and had received previous radiation therapy and/or chemotherapy. Whether this represents a different mechanism of toxicity for each of these agents or merely reflects a sampling artifact in this small series cannot be answered by our data, but it would appear that the incidence of significant toxicity would not in itself contraindicate the use of this combination in patients with head and neck cancer with such a grave prognosis

The response rate for these patients was somewhat disappointing considering the potency of the agents employed, but it must be considered in terms of the population treated. All of the patients had advanced disease that was not amenable to potentially curative radiation therapy or surgery, many were quite debilitated, and most had been treated previously with radical surgery and/or radiation therapy. It is well-known that radical surgery and radiation therapy significantly limit the possibility of obtaining responses with subsequent chemotherapy for head and neck cancer (1,14). Thus, the relatively poor response to this combination should not be taken as a contraindication to its use in patients with this disease who would be more likely to respond to chemotherapy, ie, patients who have not been previously treated. In terms of evaluating the patients who did respond, it is of interest that although the duration of response is encouraging, no clear-cut superiority to previously studied single or combination agents was demonstrated (1).

TABLE 5.—Response

No. of evaluable patients	No. of CRs	Duration of CRs (mos)	No. of PRs	Duration of PRs (mos)
29	3 (10%)	5+, 14, 7+	4 (14%)	14, 4+, 5+, ?*

*1 patient was treated with radiation therapy after obtaining a PR; he is now in CR at 16 mos.

In conclusion, we have evaluated a potent combination chemotherapy regimen in patients with far-advanced head and neck cancer. The toxicity of this regimen is acceptable in patients with these high-risk diseases, and responses of surprisingly long duration were noted. This chemotherapy regimen would appear to have a risk/benefit ratio sufficiently favorable to allow its use in randomized clinical trials, particularly in the untreated patient with head and neck cancer.

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Chemoimmunotherapy (DTIC and *Corynebacterium parvum*) as Adjuvant Treatment in Malignant Melanoma [1]

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SUMMARY

Sixty-one patients with stage I or II (lymph node involvement) malignant melanoma were prospectively randomized into a control group receiving surgical treatment only (consisting of wide excision of primary and regional node dissection) and a group receiving the same surgical treatment plus adjuvant therapy with DTIC and *Corynebacterium parvum*. Followup times ranged from 1½ to 4 years. Among the 29 patients in the surgical control group, there were five hematogenous recurrences and one regional recurrence and four patients have died. Among the 32 patients in the treatment group, there were 13 hematogenous recurrences and two regional recurrences and ten patients have died. However, the control group had six patients with involved lymph nodes while the treatment group had 15 patients in this category. It appears that although the combination of DTIC and *C. parvum* was well-tolerated, it did not reduce the recurrence and mortality rates.

[Cancer Treat Rep 63:1739–1743, 1979]

Clinical stage I malignant melanoma treated with surgery alone has a 5-year survival rate of 52.3% (1) to 76% (2). Although the 10-year survival rate may be only about 40% (3), 5-year survival rates of nearly 70% have been frequently noted (4,5). Higher rates have also been reported, usually associated with additional therapeutic modalities (6) and with groups having better prognoses, such as extremity lesions (7). Prophylactic regional node dissection apparently does not improve recurrence or survival rates (8). Considering the high rates of recurrence, the need for an effective adjuvant treatment becomes obvious.

This report describes the experience in 61 patients with malignant melanoma admitted to the Roswell Park Memorial Institute from January 1975 to June 1977.

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MATERIALS AND METHODS

Patients with malignant melanoma (Clark's level III, IV, and V) with or without involved regional lymph nodes were admitted to the study. Patients with localized primary melanoma were considered stage I and those with metastases to regional lymph nodes were considered stage II (9,10). Tests to determine metastatic spread consisted of chest roentgenograms, upper gastrointestinal and small bowel series, and liver, brain, and bone scans to rule out the presence of metastatic disease.

Skin tests were performed in all patients at the time of admission and every 2 months thereafter, with dinitrochlorobenzene 100 and 2000, Varidase, purified protein derivative, *Monilia*, and dermatophytin.

An equal number of control and treatment cards were placed in envelopes and the envelopes were mixed. Sixty-one patients were prospectively randomized in two groups by pulling an envelope for each patient. The surgical control group (29 patients) had wide excision of the primary tumor, with a 5-cm margin in most cases and split-thickness skin graft, and regional node dissection. The treatment group (32 patients) had the same surgical treatment and, in addition, chemoimmunotherapy with DTIC and *Corynebacterium parvum*. Node dissection

was not done in two patients in the control group and in one patient in the treatment group, all three having clinically negative nodes.

DTIC was given at a dose of 250 mg/m^2 iv daily × 5. *C. parvum* was given simultaneously in escalating doses of 0.7, 1.4, 2.8, 3.5, and 3.5 mg for 5 days respectively. The dose of *C. parvum* was escalated provided that the temperature elevation on the preceding day did not exceed 103°F, was maintained at the same level (for temperature elevation of 103°F–104°F), and was halved for temperature elevation of > 104°F. Burroughs Wellcome *C. parvum* was used (7 mg dry weight/ml of suspension of washed, formalin-killed organisms in 0.9% NaCl solution containing 0.01% thiomersal). The treatment was repeated at 4-week intervals for a total of 12 courses, although not all patients received this number of courses, as detailed below.

The mean followup for the control group is 19 months (median, 18) and the mean followup for the treatment group is 21.1 months (median, 17).

Patients were followed at monthly intervals for the first year of the study and at 2-month intervals for the second and third years. There were 33 males and 28 females; 60 patients were white and one was black. The mean age was 48.5 years and the median age was 53 years (range, 18–72).

No significant differences could be found between the control and treatment groups in relation to Clark's level or location of the primary site, age, or sex (table 1). However, there was a difference of borderline significance (Yates-corrected chi-square 3.22, $0.05 < P < 0.10$) in relation to histologic involvement of lymph nodes in favor of the control group (table 1).

TABLE 1.—Adjuvant chemoimmunotherapy for malignant melanoma

Study parameter	No. of patients: Surgical control group (total: 29 patients)	No. of patients: DTIC + *C. parvum* group (total: 32 patients)
Sex		
Male	16	17
Female	13	15
Primary lesion (Clark's level)		
III	11	15
IV	17	15
V	1	2
Primary tumor site		
Extremity	9	11
Other	20	21
Pathologic stage I	23 (2)*	17 (1)*
Recurrence		
Regional	—	2
Hematogenous	3 (2)†	3 (3)†
None	20	12
Present status‡		
AND	21	15
DWD	2	2
AWD	—	—
UNK	—	—
Pathologic stage II	6 (2)*	15 (8)*
Recurrence		
Regional	1	—
Hematogenous	2 (2)†	10 (5)†
None	3	5
Present status		
AND	4	4
DWD	2	8
AWD	—	2
UNK	—	1

*Stage I: Nos. in parentheses = No. of patients with clinical stage I who did not have elective node dissection; stage II: Nos. in parentheses = No. of patients with clinically palpable as well as histologically positive nodes.

†Nos. in parentheses = No. of patients with hematogenous as well as regional recurrences.

‡AND = alive, no disease; DWD = dead due to melanoma; AWD = alive with metastatic melanoma; UNK = unknown.

RESULTS

Chemoimmunotherapy with DTIC and *C. parvum* was well-tolerated. The usual subjective manifestations of DTIC treatment, nausea and vomiting, were not considerably augmented by the addition of *C. parvum*. Chills and fever were noted in the majority of patients.

One regional and five systemic recurrences occurred in the surgical control group, while two regional and 13 systemic recurrences occurred in the treatment group (borderline significance; Yates-corrected chi-square 3.53, $0.05 < P < 0.10$). Four patients in the control group died of metastatic melanoma while ten patients in the treatment group died of metastatic melanoma (table 1). One of the five patients in the control group with systemic recurrence had a single distant subcutaneous, apparently hematogenous metastasis which was removed and she has remained recurrence-free for 2 years. One of the 13 patients in the treatment group with hematogenous spread had a single visceral metastasis removed and he has also remained recurrence-free for 2 years. Two of the hematogenous recurrences in the stage I group of surgical control patients occurred in patients who did not have elective node dissection and, therefore, their histologic status remains unknown.

When stage I and II patients in both the surgical control and treatment groups were analyzed separately, no favorable effect could be discerned in either stage by comparing the treatment group to the control group (table 1).

Chemoimmunotherapy was well-tolerated. Only one patient presented with hyperpyrexia (fever > 105°F) and

TABLE 2.—Side effects of DTIC-*C. parvum* and results

Side effects	No. of patients	No. of patients failing treatment
Overall*		
None	5	3
Mild	12	6
Moderate	15	6
Fever		
< 101°F	8	4
101°F–102°F	20	10
> 102°F	4	1
Wbc count (cells/mm³)		
> 6000	13	6
3500–6000	19	9
2000–3499	0	0

*Includes nausea and vomiting, chills, and malaise.

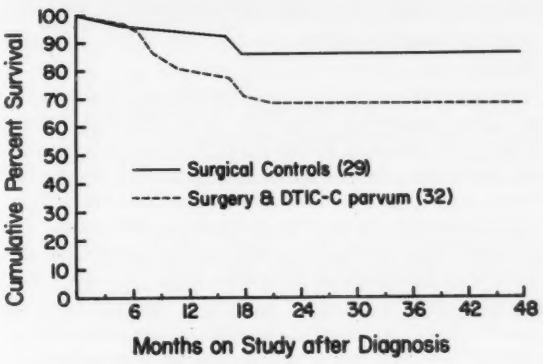

FIGURE 1.—Survival after diagnosis of malignant melanoma. Nos. in parentheses = No. of patients.

became hypotensive, but the patient responded promptly to corticosteroids and iv fluid resuscitation. In retrospectively analyzing the effects of chemoimmunotherapy and possible correlations with the results, patients were distinguished into those with average temperatures < 101°F, between 101°F and 102°F, and > 102°F for the duration of the chemoimmunotherapy course. The side effects of nausea and vomiting, chills, and malaise were distinguished into mild, moderate, and severe. The patients were also separated into three groups according to the wbc count response to treatment: those with wbc counts > 6000/mm³, those with wbc counts between 3500 and 6000/mm³, and those with wbc counts between 2000 and 3499/mm³ (table 2).

The patients in the treatment group who failed treatment had an average of 5.7 courses prior to failure. The 17 patients in the treatment group without recurrence had an average of 7.8 courses of treatment. In the treatment group there was no correlation between the number of treatments, fever response or intensity of other side effects, or absolute wbc counts (during treatment with DTIC and *C. parvum*) and the recurrence or mortality rates (table 2).

In both the treatment and control groups the patients who had recurrence had an overall median survival of 15.6 months (fig 1). Considered separately, the median survival after the first recurrence was 6 months for patients in the control group who failed and 6.2 months for patients in the treatment group who failed. The mean interval until recurrence was 6.4 months for patients in the control group who failed and 6.43 months for patients in the treatment group who failed (fig 2).

No correlation could be established between skin tests and the evolution of the disease in either group of patients.

DISCUSSION

Malignant melanoma has a significant recurrence rate after surgical treatment of the primary lesion, with the 5-year survival rate generally reported to be 60%–70% (4). Therefore, adjuvant treatment becomes necessary. DTIC has been the most extensively studied single agent in malignant melanoma, with a response rate of 21%–25% (11,12), a complete remission rate of 5% (13), and a duration of objective response of 5–6.7 months (13). However, in prospectively randomized studies DTIC used as adjuvant treatment has not improved results compared to surgical controls (14). Immunologic factors have long been recognized in patients with malignant melanoma and have raised the possibility of using immunotherapy (15). Intralesional bacillus Calmette-Guérin (BCG) in nonanergic patients with superficial lesions has been found to be effective (16). However, its value as adjuvant

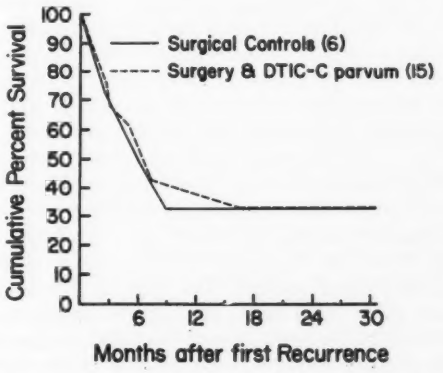

FIGURE 2.—Survival after first recurrence. Nos. in parentheses = No. of patients.

treatment remains controversial, since some studies have reported beneficial effects (17–19) while others have not (20,21).

Immunotherapy with *C. parvum* also apparently does not affect recurrence rates (22), while in another study of stage II malignant melanoma BCG immunotherapy or treatment with transfer factor did not result in significant differences compared with historical controls (23). In a randomized clinical trial using Pasteur BCG versus *C. parvum* following surgical excision of all known disease, there was no difference in relapse rates (24). Immunotherapy with "unblocking" plasma in addition to BCG scarification (Tice strain) in patients with regional lymph node involvement had results similar to those seen after BCG immunotherapy alone (25).

Chemoimmunotherapy for disseminated malignant melanoma using DTIC and actinomycin D with methanol extracted residue of BCG added little to the response rate compared with a concomitant randomized group that received the same chemotherapy alone (26). However, in another series, chemoimmunotherapy (DTIC plus BCG) appeared to improve results in advanced disease (27). A preliminary report indicated that adjuvant chemoimmunotherapy following lymph node dissection for positive nodes gave superior results when viral oncolysates of cultured allogeneic melanoma cells (infected with PR8 influenza A virus) were used as the immunotherapeutic component rather than BCG (Chicago strain) (28).

In a recent trial of adjuvant therapy by Wood et al (29), a statistically significant difference was found in favor of the chemoimmunotherapy arm with DTIC and BCG.

In the present series, 61 patients were randomized between a surgical control group and a treatment group which received the same surgical treatment plus chemoimmunotherapy with DTIC and *C. parvum*. The two groups had comparable primary lesions according to Clark's levels. However, six of 29 patients in the control group, compared to 15 of 32 in the treatment group, had melanoma metastatic to the regional nodes. Six patients had recurrence (five with hematogenous spread) in the control group compared to 15 patients (13 with hematogenous spread) in the treatment group ($0.05 < P < 0.10$). However, the overwhelming factor which determined recurrence was involvement of lymph nodes. There were six recurrences among 37 patients with known histologically negative nodes, while there were 13 recurrences among 21 patients with positive nodes (Yates-corrected chi-square 10.71, $P < 0.001$). The DTIC-*C. parvum* combination did not prolong the disease-free interval in the patients in the treatment group who failed, compared to the disease-free interval in the patients in the control group who failed, nor did it prolong their survival after recurrence. When the data were analyzed by stage, this combination appeared to be ineffective for both stage I and II patients.

The recurrence and death rates in the two groups correlate with the incidence of lymph node involvement. The latter was approximately two and one-half times higher in the treatment group than in the surgical control group, as were the recurrence and death rates. The majority of patients with hematogenous recurrence (ten of 13) in the treatment group were also in the group of patients with involved nodes.

Of the group of eight patients with clinically and histologically positive nodes treated with DTIC and *C. parvum*, there is only one survivor free of disease. This survival rate is certainly not better than the 5-year survival rate for surgical historical controls with palpable positive nodes (4,30).

The present study points out the importance of stratifying patients according to lymph node status to avoid dissimilarities in the studied group. The DTIC-*C. parvum* combination as given in this study did not decrease recurrence and mortality rates in stage I or II malignant melanoma.

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Advanced Ovarian Carcinoma: A Pilot Study of *cis*-Dichlorodiammineplatinum(II) in Combination With Adriamycin and Cyclophosphamide in Previously Untreated Patients and as a Single Agent in Previously Treated Patients [1]

C. J. Williams,* K. E. Stevenson, R. B. Buchanan, and J. M. A. Whitehouse [2,3]

SUMMARY

Twenty-eight patients with ovarian carcinoma (FIGO stage III or IV) were treated with *cis*-dichlorodiammineplatinum(II) (CDDP). Previously untreated patients received a combination (PACe) of CDDP (80 mg/m² iv Day 1), Adriamycin (40 mg/m² iv Day 1), and cyclophosphamide (200 mg/m² orally Days 2–5). Previously treated patients received CDDP (80 mg/m² Day 1) alone. The first 20 patients (63 courses) were given CDDP randomly by bolus injection or 24- or 48-hour infusion, and plasma and urinary levels of total CDDP were measured by atomic absorption spectrometry. Following bolus CDDP, plasma levels decayed in a biphasic manner (initial half-life, 30 minutes; secondary half-life, 3–4 days) and urinary excretion was incomplete (20% at 24 hours and 30% at 48 hours). After 24- and 48-hour infusions, peak levels of CDDP were reached at the end of the infusion and excretion was incomplete (14% at 24 hours and 21% at 48 hours). Low levels of CDDP were detected in the urine of patients 4 weeks after treatment. Serial renal studies showed a progressive reduction in renal function although renal failure was not seen; this finding was unrelated to the mode of CDDP administration. Nausea and vomiting were seen in all patients and were prolonged in those receiving infusions of CDDP. Sixteen patients received PACe; 14 have completed five cycles with 11 complete remissions and three partial remissions and two received one cycle and then refused further therapy. Four deaths have occurred in this group (two patients who refused further therapy, one who had partial remission, and one who had complete remission), one patient has relapsed after complete remission and is receiving further chemotherapy, and the rest continue in complete remission (3+ to 13+ months). Of 12 previously treated patients, four have achieved partial remission with CDDP alone and are alive; seven of eight nonresponders have died. PACe is an effective combination for inducing remissions in advanced ovarian carcinoma and will now be compared with single alkylating agent therapy in a randomized trial. Twenty-four- and 48-hour infusions did not reduce the toxicity of PACe and were not apparently associated with greater antitumor effects.

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Single alkylating agent chemotherapy has been the treatment of choice for advanced ovarian carcinoma for the past 20 years. Recently, reports from several nonrandomized series have claimed improved results with combination chemotherapy (1,2), and a new combination (Hexa-CAF)[4] has been shown to be superior to melphalan in a randomized study (3).

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[4] Hexa-CAF = hexamethylmelamine, cyclophosphamide, methotrexate, and 5-fluorouracil.

One of the more active of these combinations is Adriamycin plus cyclophosphamide; Lloyd et al (2), using this combination, reported that 14 of 23 (61%) patients with stage III and IV ovarian carcinoma achieved an objective response. In previously untreated patients the response rate was 80% (12 of 15 patients) with 33% (five of 15 patients) achieving a complete clinical remission.

Edmonson et al (4) found, in a randomized study, that Adriamycin and cyclophosphamide were more effective than cyclophosphamide alone for limited disease, but similar results were not obtained in patients with more extensive disease. Turbow et al (5), in a small randomized study, showed an improved remission rate for Adriamycin and cyclophosphamide in comparison with cyclophosphamide alone. The survival curves for the two groups were, however, similar. *cis*-Dichlorodiammineplatinum(II) (CDDP) is a new drug which has been shown to be useful in advanced ovarian carcinoma resistant to alkylating agent therapy (6).

A pilot study of CDDP in combination with Adriamycin and cyclophosphamide (PACe) in patients who had not previously received chemotherapy was begun in September 1977. A second study of CDDP as a single agent in patients resistant to conventional alkylating agent therapy was carried out concurrently.

CDDP produces profound nausea and vomiting in addition to renal and audiologic toxicity when given by iv bolus or short-duration infusion, and the pharmacokinetics and toxicity of CDDP given by bolus injection and 24- and 48-hour infusion were studied.

PATIENTS AND METHODS

Twenty-eight patients with advanced ovarian carcinoma (FIGO stage III or IV) were admitted to the studies between September 1977 and September 1978. All patients had ovarian carcinoma of epithelial origin; the patient characteristics are shown in table 1. All previously untreated patients had bulky disease, which was palpable in the abdomen in 13 of 16. The surgery that these patients underwent did not reduce the tumor bulk to < 2 cm and these patients formed a poor prognostic group. All patients in the previously treated group had palpable disease. Patients who had not received prior chemotherapy were treated with PACe (fig 1). Courses of PACe were repeated every 4 weeks for a total of five cycles and patients were then re-assessed clinically; those in clinical complete remission started maintenance therapy with chlorambucil. Partial responders and nonresponders received further chemotherapy at the discretion of the investigator. Patients who had received previous chemotherapy were treated with CDDP alone (fig 1) every 3–4 weeks.

Prehydration with dextrose/saline was used for at least 2 hours prior to CDDP in both regimens (fig 2), CDDP being given when the urine output reached 150 ml/hour. Furosemide (40 mg iv) was given with the bolus doses of CDDP, and iv fluids were continued to maintain urine output at > 150 ml/hour as long as vomiting persisted. Patients receiving 24- or 48-hour infusions only received diuretics if the urine output fell to < 150 ml/hour. Infusion rates were high (4–6 liters/24 hours) to maintain urine output and frequent electrolyte studies were obtained because of the risk of hyponatremia. CDDP was not infused in dextrose since this is reported to cause drug instability (7).

TABLE 1.—Patient characteristics

Characteristic	Treatment group: PACe	Treatment group: CDDP alone
No. of patients	16	12
Mean age in yrs (range)	49 (36–68)	52 (40–67)
FIGO stage		
III	10	8
IV	6	4
Histologic type		
Serous	8	8
Mucinous	2	2
Undifferentiated	6	2
Operation*		
Biopsy only	5	—
Excision of masses	7	—
TAH + BSO	3	—
TAH + BSO + omentectomy	1	—
Residual postoperative tumor		
Palpable in abdomen	13	—
> 2 cm, not palpable	3	—
< 2 cm	0	—

*TAH = total abdominal hysterectomy; BSO = bilateral salpingo-oophorectomy.

All patients who had not previously received chemotherapy had normal renal function (creatinine clearance ≥ 100 ml/minute) prior to treatment. Those who had received prior chemotherapy had a creatinine clearance > 70 ml/minute. The creatinine clearance was measured before each cycle and CDDP was omitted if the value fell to < 70 ml/minute. Pretreatment audiograms, full blood cell count, biochemical screen, abdominal and pelvic ultrasonograms, and chest roentgenogram were obtained. Hematologic and biochemical tests were repeated before each cycle and audiograms and ultrasonograms were repeated after the third and fifth cycles.

After five cycles of chemotherapy all patients were restaged by physical examination, ultrasonography, radiology, and any other relevant investigations. A complete remission was defined as complete clinical disappearance of all evidence of disease (short of lapa-

1. PACe
   cis-Dichlorodiammineplatinum(II): 80 mg/m^2 iv on Day 1†
   Adriamycin: 40 mg/m^2 iv on Day 1
   Cyclophosphamide: 200 mg/m^2 orally on Days 2–5

   Course repeated every 4 wks

2. cis-Dichlorodiammineplatinum(II) alone
   80 mg/m^2 iv on Day 1†

   Course repeated every 3–4 wks

†cis-Dichlorodiammineplatinum(II) was given randomly as a bolus injection or as a 24- or 48-hr infusion to the first 20 patients. Subsequent patients received bolus injections.

FIGURE 1.—Chemotherapy regimens for advanced ovarian carcinoma.

roscopy or laparotomy) for at least 1 month. A partial remission was defined as a ≥ 50% reduction in the products of the diameters of all measurable lesions coupled with disappearance of ascites for at least 1 month. Survival was calculated from the start of the first cycle of chemotherapy, and duration of complete remission was calculated from the completion of the fifth cycle of chemotherapy and restaging.

Serial specimens of blood and urine were collected during 63 courses given to the first 20 patients treated. These samples were aliquoted, stored in liquid nitrogen, and assayed in batches. Plasma and urine concentrations of platinum were measured by atomic absorption spectrometry (8) (Perkins-Elmer 360 with heated graphite atomizer HGA 74). Plasma and urine was not predigested but required dilution with distilled water for adequate atomization (9). All samples were measured in triplicate; reproducibility was ± 2.5%.

CLINICAL RESULTS (table 2)

Of the 16 patients who had not received prior chemotherapy, 14 have completed five cycles of PACe. Eleven of these achieved a complete clinical remission and three achieved a partial remission. Two patients received one cycle of chemotherapy only and refused further treatment. One had severe vomiting following CDDP and the second had an epileptic fit due to hyponatremia; neither received further chemotherapy. Survival for the entire group is shown in figure 3.

Twelve patients who failed prior chemotherapy have completed therapy with single-agent CDDP. Four achieved an objective partial remission and eight failed to respond to CDDP. Survival for this group is shown in figure 3.

Four deaths have occurred in the PACe group. Both patients who received only one cycle of chemotherapy died within 3 months and one patient who achieved a partial remission relapsed and died 3 months after starting maintenance chlorambucil. One patient who achieved a complete clinical remission relapsed after 3 months on maintenance therapy and died 2 months later. Both patients who developed progressive disease after completing five cycles of PACe were resistant to alternative chemotherapy. Of the remaining ten patients who achieved complete remission, nine continue in remission at 3+ to

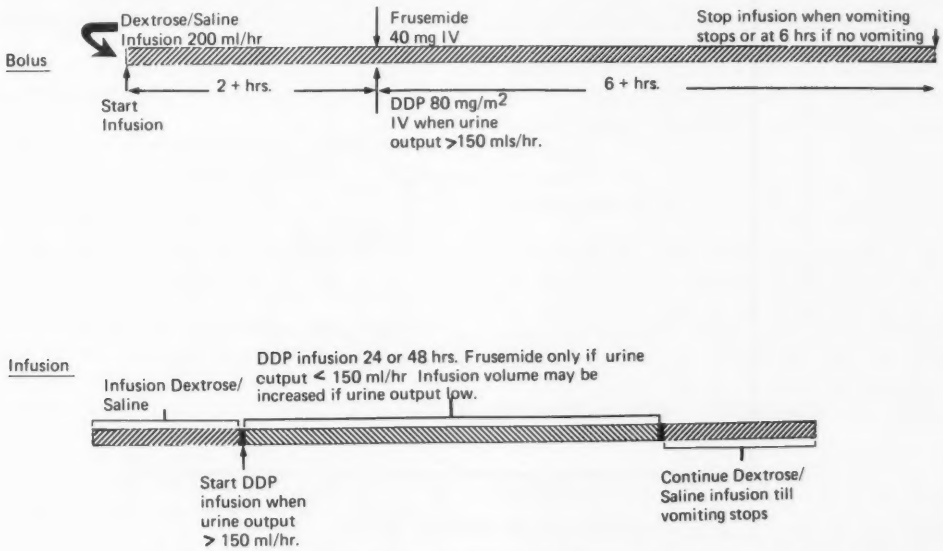

FIGURE 2.—Hydration regimen for CDDP.

TABLE 2.—Clinical responses

Treatment group	No. of patients	No. of complete remissions (duration in mos)	No. of partial remissions (duration in mos)	No. of patients with no response or progressive disease, or who refused chemotherapy	No. of deaths
PACe	16	11 (69%) (3.2,* 3.6, 5.6, 7.7, 8.0, 8.1, 8.7, 9.2, 10.0,* 11.2, 11.9)	3 (19%) (2.0,* 3.0, 3.2)	2 (12%)	4 (2 received 1 cycle only, 1 had partial remission, 1 had complete remission)
CDDP alone	12	—	4 (33.3%)	8 (66.7%)	7 (all nonresponders)

*Relapsed.

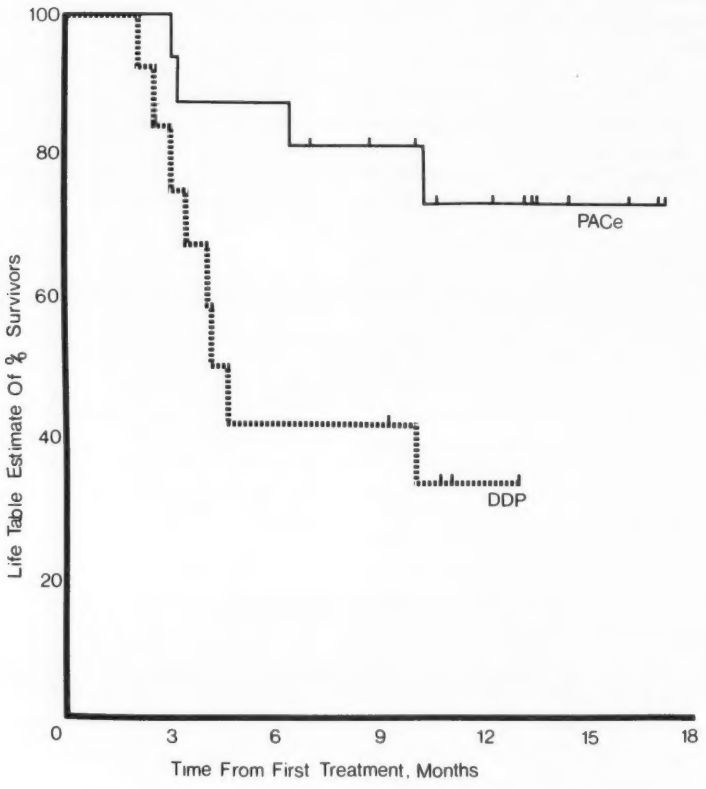

FIGURE 3.—Survival curves for patients treated with PACe or CDDP alone.

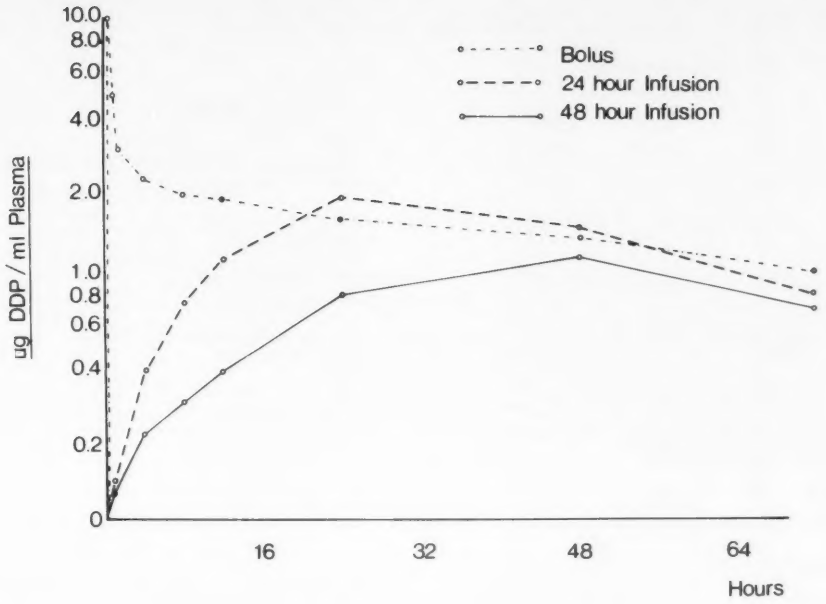

FIGURE 4.—Plasma levels of CDDP.

13+ months. One patient has relapsed at 10.0 months and is receiving further chemotherapy.

The four previously treated patients who attained a partial remission remain alive but seven of eight of the nonresponders have died.

PHARMACOKINETICS

Eleven patients (24 courses) received CDDP by bolus, ten patients (21 courses) received CDDP by a 24-hour infusion, and nine patients (18 courses) received CDDP by a 48-hour infusion. After iv bolus administration CDDP plasma levels decayed in a biphasic manner (fig 4). The initial plasma half-life was 30 minutes and the secondary plasma half-life was 3–4 days. Urinary excretion of CDDP (fig 5) was incomplete, only 20% being excreted at 24 hours and 30% at 48 hours. Peak CDDP levels were about 10 μg/ml, although levels of free CDDP were not determined.

Following CDDP administration by 24- or 48-hour infusion peak plasma levels were reached at the end of the infusion (fig 4). Urinary excretion was again incomplete: 14% at 24 hours and 21% at 48 hours (fig 5). Low levels of CDDP ($\leq$ 0.2 μg/ml) were found in the urine of patients who had estimations immediately prior to their next cycle of CDDP.

TOXICITY

Renal

Renal function as measured by the mean creatinine clearance, serum creatinine, and blood urea showed progressive deterioration in patients receiving more than three cycles of CDDP (table 3). No episodes of acute failure occurred despite the gradual deterioration in renal function.

Gastrointestinal

Assessment of the intensity of vomiting was based on the frequency of vomiting recorded on fluid charts and by personal assessment by one of the authors. Nausea and vomiting were universal although of varying severity. Twenty-four- or 48-hour infusions of CDDP did not reduce the intensity of vomiting but resulted in more prolonged vomiting which became unacceptable in some patients (table 4).

Hematologic

Leukopenia and thrombocytopenia were mild in previously untreated patients and there were no episodes of sepsis or bleeding related to marrow suppression (table

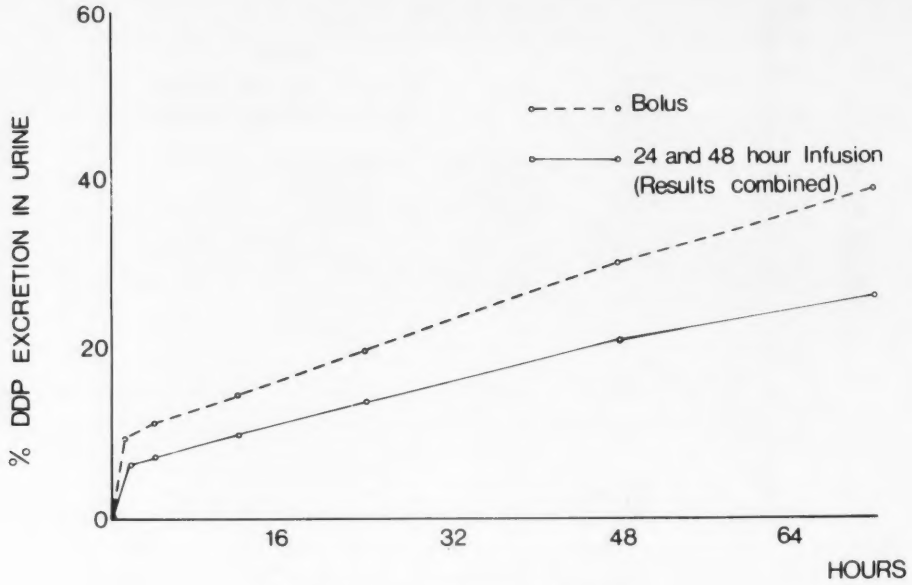

FIGURE 5.—Urinary excretion of CDDP.

TABLE 3.—Renal toxicity (121 courses in 28 patients)

	Treatment group	Cycle No. 0	1	2	3	4	5
Mean creatinine clearance (ml/min) (± SD)	No previous chemotherapy	114 (± 16)	114 (± 14)	96 (± 20)	103 (± 13)	96 (± 12)	78 (± 10)
	Previous chemotherapy	98 (± 14)	88 (± 12)	80 (± 15)	76 (± 13)	87 (± 20)	80 (± 44)
Mean plasma creatinine (mg/100 ml) (± SD)	No previous chemotherapy	0.9 (± 0.1)	0.9 (± 0.1)	1.0 (± 0.2)	1.1 (± 0.2)	1.2 (± 0.2)	1.3 (± 0.2)
	Previous chemotherapy	1.0 (± 0.2)	1.0 (± 0.2)	1.0 (± 0.2)	1.1 (± 0.2)	1.2 (± 0.3)	1.4 (± 0.4)
Mean blood urea (mg/100 ml) (± SD)	No previous chemotherapy	25.0 (± 6)	26 (± 5)	30.0 (± 8)	34.0 (± 7)	38.0 (± 10)	40.0 (± 7)
	Previous chemotherapy	30 (± 6)	32 (± 5)	35 (± 7)	35 (± 8)	38 (± 9)	41 (± 12)

5). Patients who had received previous chemotherapy had, as expected, greater marrow suppression despite receiving CDDP only. Platelet counts of $< 100{,}000/mm^3$ occurred in three of 12 patients, although no episodes of bleeding or sepsis occurred (table 5).

Increasing anemia was common, regardless of previous treatment, and 60% of patients receiving five complete cycles of therapy required at least one transfusion (table 5).

Audiologic

Two patients who had received five cycles of CDDP developed unilateral tinnitus and high-tone hearing loss confirmed by audiology. They both had normal renal function and no predisposing factor was identified. No clinical or audiologic abnormalities were detected in the remaining patients.

Neurologic

One patient developed a stocking and glove distribution peripheral neuropathy with associated loss of tendon reflexes. This has gradually improved over a 3-month period.

Another patient had an epileptic fit shortly after completing chemotherapy and was found to be profoundly

TABLE 4.—Nausea and vomiting (63 courses in 20 patients)

Grade	Definition	No. of courses with toxicity/No. given by— Bolus injection	24-hr infusion	48-hr infusion
1	No nausea or vomiting	—	—	—
2	Nausea and vomiting controlled by routine antiemetics	19/24 (80%)	3/21 (14%)	3/18 (17%)
3	Severe nausea and vomiting requiring regular antiemetics	5/24 (20%)	18/21 (86%)	9/18 (49%)
4	Severe nausea and vomiting uncontrolled by antiemetics	—	—	3/18 (17%)
5	Severe nausea and vomiting requiring cessation of therapy	—	—	3/18 (17%)

TABLE 5.—Hematologic toxicity (121 courses in 28 patients)

	Treatment group	Cycle No. 0	1	2	3	4	5
Mean wbc count (cells × 10^3/mm^3) (± SD)	No previous chemotherapy	10.6 (± 3.2)	8.5 (± 3.4)	6.3 (± 1.6)	4.9 (± 1.4)	4.5 (± 1.4)	4.2 (± 1.4)
	Previous chemotherapy	5.2 (± 1.3)	5.3 (± 1.8)	5.1 (± 1.6)	4.6 (± 1.1)	4.4 (± 1.7)	4.1 (± 1.3)
Mean hemoglobin (g/100 ml) (± SD)	No previous chemotherapy	13.1 (± 1.5)	13.0 (± 1.4)	11.8 (± 1.4)	9.6 (± 3.2)	10.0 (± 0.9)	10.5 (± 0.8)
	Previous chemotherapy	11.8 (± 1.2)	10.5 (± 1.2)	10.6 (± 1.7)	11.4 (± 1.6)	10.8 (± 1.8)	10.0 (± 1.2)
Mean platelet count (cells × 10^3/mm^3) (± SD)	No previous chemotherapy	348 (± 179)	321 (± 191)	261 (± 96)	230 (± 114)	224 (± 71)	196 (± 76)
	Previous chemotherapy	237 (± 104)	233 (± 98)	165 (± 78)	159 (± 54)	153 (± 63)	142 (± 53)

hyponatremic. Hyponatremia was caused by a combination of fluid overload and concurrent paracentesis of 4L ascites. Fits were controlled with iv diazepam and the correction of electrolyte imbalance.

Other Toxic Effects

One patient had hemorrhagic cystitis presumed related to cyclophosphamide, although pelvic irradiation (4750 rads in 19 fractions over 38 days) had been administered 2 months prior to starting chemotherapy.

All patients developed total alopecia as anticipated; there were no cases of Adriamycin-associated cardiac failure. No allergic episodes were noted.

DISCUSSION

Despite encouraging single-agent response rates with several drugs, long-term survival in patients with advanced ovarian carcinoma remains uncommon. It has been suggested that the combination of Adriamycin and cyclophosphamide (2,10) is more active than single-agent therapy, and CDDP has been shown to be an active agent in patients failing previous chemotherapy (6). A combination of these three drugs was, therefore, selected for this pilot study.

The response rate in this study for patients who had not received prior chemotherapy (overall rate, 87.5%) is higher than any reported with single alkylating agent therapy and comparable with the best results achieved with combination chemotherapy. Also encouraging is the very high complete remission rate (69%) achieved after five cycles of PACe. Although many studies of single alkylating agents used clinical criteria for assessing tumor response, more recent studies of combination chemotherapy have used surgical restaging. Responses in this study were judged by clinical restaging and a number of patients in complete clinical remission would undoubtedly

have been shown to have residual disease after surgical staging (one patient relapsed only 3 months after attaining a clinical complete remission). However, such surgical restaging would not have reduced the high overall remission rate seen in this group of patients with advanced disease. The followup of this pilot study is so short that the potential for long-term survival among those achieving a complete remission cannot be judged and the data can only be used to suggest formal randomized studies to test the effectiveness of such combinations.

CDDP causes renal toxicity, which may be related to peak plasma levels (11). In addition, CDDP causes severe vomiting which may be dose-limiting (12). A study of CDDP given by bolus injection and 24- or 48-hour infusion was, therefore, included in the study design to see if infusions would reduce the toxicity.

The pharmacokinetic study of bolus CDDP produced results similar to those previously reported (13,14), with a biphasic plasma decay curve and incomplete CDDP excretion. Measurement of free CDDP, however, suggests that this biphasic excretion pattern is a result of protein binding of CDDP (15,16).

The plasma concentration of CDDP after 24- and 48-hour infusions reached a peak level at the end of the infusion and there was a slow and incomplete urinary excretion. The peak levels of CDDP achieved after 24- and 48-hour infusions were 20% and 10% of those seen after a single bolus of the same dose. If the renal and emetic toxic effects of CDDP are related to the peak plasma level, as suggested (11), such effects might have been expected to be abolished by the use of 24- and 48-hour infusions in this study. In fact, this was not the case; cumulative renal toxicity was seen in patients receiving CDDP by both bolus injection and 24- or 48-hour infusion, and the duration of nausea and vomiting was increased by the infusions.

A trend of worsening renal function (measured by creatinine clearance, plasma creatinine, and blood urea) was seen with successive cycles of CDDP and clearly suggests cumulative renal toxicity. This toxicity appears to be permanent (17,18) and no improvement in creatinine clearance has been seen in our patients up to 1 year after stopping CDDP.

Nausea and vomiting remain major problems with CDDP. Not only did infusions of CDDP fail to reduce the intensity of nausea and vomiting, they also resulted in a prolongation of the emetic effect which then became unacceptable, causing the CDDP infusion to be stopped early in two patients receiving a 48-hour infusion. More prolonged infusions (5 days) have been reported to be less emetic, although nausea and vomiting still occur during therapy (19).

Hematologic toxicity was relatively mild with this combination, although anemia was common and necessitated transfusions in 60% of patients receiving five cycles of PACe. Single instances of tinnitus and hearing loss, peripheral neuropathy, and a seizure due to hyponatremia were uncommon side effects previously associated with CDDP.

PACe appears to be a highly active combination, and two studies (20,21) using the same three drugs (in lower doses and by a different schedule) have also shown encouraging response rates. A randomized study comparing PACe with chlorambucil has therefore begun. CDDP will be given by bolus injection since it is more convenient and since this study has clearly demonstrated that this mode of administration causes less nausea than 24- or 48-hour infusions. Complete remissions will be confirmed surgically in all cases and surgical debulking (22) will be performed in those patients with resectable disease at restaging. Patients with a surgically confirmed complete remission will be further randomized for maintenance therapy.

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Phase II Study of VP-16-213 in Childhood Malignant Disease: A Children's Cancer Study Group Report[1]

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SUMMARY

VP-16-213, a semisynthetic podophyllotoxin, was tested for antitumor and clinical toxicity in 126 children. The drug was administered iv daily × 5 days every 2 weeks at a starting dose of 75 mg/m²/day. The dose was increased by 25 mg/m²/day/course until clinical response or significant toxicity occurred. The only major toxicity was hematologic, with neutropenia as the most predominant feature. There was one local allergic reaction at the site of injection. No systemic allergic responses were reported. The drug demonstrated significant activity in acute myelomonocytic leukemia with four responses among 19 patients, less activity in acute myelocytic leukemia with two responses among 44 patients, and little activity in acute lymphocytic leukemia with only one partial response among 12 patients. Objective partial responses occurred in ten of 48 patients with solid tumors: two each with Wilms' tumor, lymphoma, and histiocytosis X, and one each with rhabdomyosarcoma, neuroblastoma, Ewing's sarcoma, and undifferentiated carcinoma. The inclusion of VP-16-213 in combination chemotherapy for childhood acute myelomonocytic leukemia and acute myelocytic leukemia appears indicated in patients relapsing after initial therapy. For solid tumors this is an interim report, with further patient accrual required before specific comments can be made.

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VP-16-213 is a semisynthetic podophyllotoxin. It is derived from a crystalline extract of the May Apple plant (1). The drug's antineoplastic properties are primarily attributed to metaphase arrest of dividing cells (2). The drug appears to have S- or G_2-phase specificity in that it also prevents cells from entering mitosis (3–6). There is evidence that suggests that VP-16-213 causes inhibition of DNA synthesis with no effect on RNA or protein synthesis (3,6).

PATIENTS AND METHODS

The study began in November 1975 and was concluded in October 1977 for entry of patients with leukemia, but is still open to selected patients with solid tumors. Patient eligibility was limited to children (< 21 years of age) with malignant disease refractory to conventional therapy, including surgery, irradiation, and standard drug or drug combinations. Only patients with measurable disease were included. The study had to be approved by institutional review committees before therapy could be utilized. Initiation of therapy was delayed in patients with severe granulocytopenia resulting from prior chemotherapy until after the absolute neutrophil count rose to > 1000/μl. There was no delay in patients with neutropenia secondary to malignant disease involving the bone marrow. Thrombocytopenia and anemia did not exclude a patient from entry in the study; platelet and rbc transfusions were administered accordingly.

Drug Administration

VP-16-213 was administered iv over 30 minutes daily × 5 days every 14 days. The initial dose was 75 mg/m²/day, and, if there was no initial toxicity, the daily dose was increased by 25 mg/m²/day/course in subsequent courses until either a response occurred or toxicity was not tolerable. The dose schedule was patterned after the results of the European Organization for Research on Treatment of Cancer study which used 60 mg/m²/day × 5 days every 2 weeks (7). It was modified further after a pilot study at the Children's Orthopedic Hospital and Medical Center, Seattle, WA, in which five consecutive patients experienced minimal toxic effects from VP-16-213 given at 75–125 mg/m²/dose × 5. In June 1976 the study was modified to start at 100 mg/m²/dose × 5 days because little toxicity and no responses were observed at the lower initial dose.

Duration of Therapy

Patients with progressive disease after two courses, or no response after three courses, were removed from the study. Patients removed from the study prior to Day 28 were included in the analysis if the disease was obviously progressive despite therapy.

Criteria for Response and Toxicity

Only responses of ≥ 4 weeks' duration were accepted. A complete response (CR) was defined as the total disappearance of all recognizable disease. Partial response (PR) was defined as a ≥ 50% decrease in the product of the perpendicular diameters of one or more measurable tumors. Static and progressive disease were defined as no response (NR). Hematologic malignancies were quantitated according to the M1, M2, or M3 classification of the leukemias. An M1 marrow is < 5% blast cells in a normocellular marrow, an M2 marrow is 5%–25% blast cells, and an M3 marrow is > 25% blast cells. If the bone marrow improved from M3 to M2, the patient was regarded as having a PR, provided the improvement lasted at least 4 weeks.

Toxicity was graded on a scale of 1–4, with grade 4 defined as life-threatening. Specific limits for each toxicity grade depended on the organ system and on the patient's age (copy available on request from the Children's Cancer Study Group). Hematologic toxicity was evaluated only in patients without tumor invasion of the bone marrow. Since many patients had alopecia prior to treatment with VP-16-213, data on this toxic effect were not recorded. Hematologic toxicity was graded as follows: *grade 1 (mild), continue drug*—absolute neutrophil count of > 1000/mm³, platelet count of > 50,000/mm³, Hgb level of > 9 g/100 ml, and marrow normal cellularity; *grade 2 (moderate), reduce drug*—absolute neutrophil count of > 500/mm³, platelet count of > 25,000/mm³, Hgb level of > 7 g/100 ml, and marrow decreased cellularity; *grade 3 (severe), stop drug*—absolute neutrophil count of ≤ 500/mm³, platelet count of < 25,000/mm³, Hgb level of < 7 g/100 ml, and marrow aplastic; and *grade 4 (life-threatening*—infection with absolute neutrophil count of < 500/mm³, hemorrhage with platelet count of < 25,000/mm³, anemia with heart failure, and marrow aplastic with infection or hemorrhage.

Required Studies

Hemograms were obtained at least twice weekly. Biweekly studies included physical examination, serum creatinine, BUN, alkaline phosphatase, bilirubin, SGOT, and urinalysis. Bone marrows, lumbar punctures, radiograms, radionuclide scans, computerized axial tomograms, catecholamine excretion, and other staging procedures, depending on the tumor type, were performed at 4-week intervals.

RESULTS

Of the 132 patients who were entered in the study, 126 are evaluable (six patients were entered by error and

TABLE 1.—Response by diagnosis in phase II study of VP-16-213

Diagnosis	Total No. of patients	No. of patients with—			No. of unevaluable patients
		CR	PR	NR	
Acute leukemia					
Acute lymphocytic leukemia	12		1	11	
Acute myelocytic leukemia	44	2	0	41	1
Acute myelomonocytic leukemia	19	3	1	14	1
Acute monocytic leukemia	2			2	
Acute erythrocytic leukemia	1		1		
Totals	78	5	3	68	2
Solid tumors					
Neuroblastoma	7		1	6	
Wilms' tumor	7		2	5	
Rhabdomyosarcoma	6		1	4	1
Ewing's sarcoma	4		1	2	1
Histiocytosis X	5		2	2	1
Osteogenic sarcoma	2			2	
Undifferentiated carcinoma	7		1	6	
Lymphoma	7	1	1	4	1
Hodgkin's disease	1			1	
Hepatoblastoma	2			2	
Totals	48	1	9	34	4

were rejected). Seventy-eight had acute leukemia and 48 had solid tumors (table 1). Many of these patients had far-advanced disease; 25 patients (20% of those entered) died of progressive disease rapidly with no response whatsoever to the initial course and 122 patients (97%) subsequently have died from progressive disease. Overall compliance with the protocol was excellent. As shown in table 1, five patients were not evaluable because of inadequate information, and in one additional patient evaluation was not possible because the drug was administered after surgical removal of a recurrence (rhabdomyosarcoma) with microscopic residual disease, leaving no measurable disease present (protocol violation); however, the patient received 20 courses of drug at 100 mg/m^2/dose × 5 days over 614 days without toxicity before the drug was discontinued. The patient is still living with no evidence of disease 715 days after starting the drug on March 5, 1976. This patient is the nonevaluable patient (rhabdomyosarcoma) shown in table 1.

Efficacy (table 1)

In acute leukemia there were five CRs and three PRs in 76 evaluable patients: one PR among 12 patients with acute lymphocytic leukemia, two CRs among 43 patients with acute myelocytic leukemia, three CRs and one PR among 18 patients with acute myelomonocytic leukemia, two NRs among two patients with acute monocytic leukemia, and one PR in a patient with acute erythrocytic leukemia. The patients who achieved a complete M1 remission were in the study for 199, 152, 106, 72, and 60 days before relapse. For those patients with a PR, the time before exacerbation of their disease was 203, 163, and 75 days. In the five patients with leukemia achieving a CR, the following doses were required: 75 mg/m^2/day in three patients (after two courses), 90 mg/m^2/day in one (after three courses), and 100 mg/m^2/day in one (after two courses). In the three patients with leukemia achieving a PR, the following doses were required: 135 mg/m^2/day in one patient (15 courses) and 175 mg/m^2/day in two (eight courses). In one of the latter patients, increasing the dose to 250 mg/m^2/day did not improve the PR.

Forty-four of 48 patients with solid tumors were evaluable for response. Only one patient achieved a CR. This patient had non-Hodgkin's lymphoma and was in the study for 92 days before exacerbation. The number of individual patients in all disease categories is small, making analysis of efficacy impossible at this time. As shown in table 1, the disease categories in which NRs were observed are: osteogenic sarcoma (two patients), Hodgkin's disease (one patient), and hepatoblastoma (two patients). Patient responses occurred in one of seven patients with neuroblastoma, in two of seven with Wilms' tumor, in one of five with rhabdomyosarcoma, in one of three with Ewing's sarcoma, in two of four with histiocytosis X, in one of seven with undifferentiated carcinoma, and in two of six with lymphoma.

In patients with non-Hodgkin's lymphomas, one CR occurred at a dose of 120 mg/m^2 and one PR occurred at

TABLE 2.—Toxicity of phase II study of VP-16-213

Toxicity	No. of patients with— Grade 0	1	2	3	4	Total No. of evaluable patients
Hematologic	26	1	12	11	0	50
Gastrointestinal	98	4	6	4	0	112
Hepatic	110	0	2	0	0	112
Neurologic	111	0	1	0	0	112
Hypotension	110	0	2	0	0	112
Bone	110	0	2	0	0	112

a dose of 100 mg/m². In patients with solid tumors, PRs occurred at a dose of 100 mg/m²/day in three patients (one with neuroblastoma and two with Wilms' tumor) and at a dose of 150 mg/m²/day in five patients (two with histiocytosis X and one each with rhabdomyosarcoma, Ewing's sarcoma, and nasal pharyngeal carcinoma).

Toxicity (table 2)

Toxicity data are available for 112 of 118 evaluable patients. Hematologic toxicity, however, is evaluable for only 50 patients: 42 with solid tumors and the eight with leukemia who achieved a response. There were no instances of life-threatening toxicity. Only 14 of 112 (13%) patients had any gastrointestinal toxicity, and in only four of 112 (3%) patients was the toxicity severe enough to have the dose decreased. Hematologic toxicity was the major problem but was not severe, with 21 of 50 (42%) patients having neutropenia severe enough to require a decrease in dose or a delay of a drug course. Only two of 112 (2%) patients had hypotension from drug administration and this was mild and had no effect on further therapy. One patient developed a swelling of the forearm and upper arm with erythema while the drug was being infused in a vein in the hand of the same arm. No generalized allergic reactions were seen in this patient or reported in any of the 112 evaluable patients.

DISCUSSION

VP-16-213 administered daily for 5 days every 2 weeks appears to be effective in advanced acute myelomonocytic leukemia, less effective in advanced acute myelocytic leukemia, and not effective in advanced acute lymphocytic leukemia. This agrees with earlier reports in adults (7–10).

In acute nonlymphocytic leukemia, 100 mg/m²/day would appear to be the top daily dose required when given for 5 days every 2 weeks for induction of remission. Higher doses did not change a PR to a CR, and doses of > 100 mg/m²/day did not increase the response rate.

Not enough patients in any single solid tumor group have been accrued at the time of this report; therefore, these observations are an interim report for this phase of the study. The following comments, however, can be made. VP-16-213 appears to be effective in non-Hodgkin's lymphoma in children, as reported by others (7,11). This study supports the suggestion that the drug is effective in Wilms' tumor (12). The agent appears disappointing for neuroblastoma.

The effective dose range for solid tumors appears to be 100–150 mg/m²/day × 5 days. Higher doses have not increased the response rate to date.

CONCLUSIONS

VP-16-213 is well-tolerated with little hepatotoxicity or renal toxicity. Hypotension has not been a problem in children. Myelotoxicity is a problem but has not been severe. Unlike the vinca alkaloids, neurologic toxicity is rare with VP-16-213.

The results of this study suggest that this agent should be utilized in combination-drug programs for the treatment of acute nonlymphocytic leukemia and that further use as a phase II agent is warranted in lymphoma, Wilms' tumor, histiocytosis X, rhabdomyosarcoma, Ewing's sarcoma, undifferentiated carcinoma, osteogenic sarcoma, and hepatoblastoma.

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Effects of Endotoxin and Extracts of Pregnant Mouse Uterus on the Recovery of Hemopoiesis After 5-Fluorouracil [1]

G. S. Hodgson* and T. R. Bradley [2]

SUMMARY

A single injection of endotoxin, 4 hours after administration of 150 mg/kg of 5-fluorouracil (5-FU), stimulated the recovery of bone marrow hemopoietic cells that form colonies in spleen (CFU-S) or in culture (GM-CFC), of erythropoiesis, and of platelet production. *Corynebacterium parvum* injections had similar effects. Endotoxin-free extracts of tissues (from pregnant mouse uterus, placenta, and embryo) which have a high content of the factor(s) capable of stimulating growth of GM-CFC in vitro also stimulated recovery of CFU-S and GM-CFC when given in single injections 4 hours after 5-FU. A striking effect of both endotoxin and tissue extracts was the acceleration of the regeneration of a particular subclass of granulocyte macrophage progenitors, those capable of forming large colonies (> 0.25 mm) in agar. The results indicate that recovery of hemopoiesis after depression by 5-FU can be stimulated not only by injection of bacterial products, but also by injection of endotoxin-free tissue extracts which contain high levels of hemopoietic regulators.

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5-Fluorouracil (5-FU) is a cytotoxic agent which preferentially kills proliferating hemopoietic cells (1). The hemopoietic stem cells (CFU-S) that survive exposure to 5-FU have a higher capacity to generate CFU-S and progenitors of granulocytes and macrophages (GM-CFC) than those of normal bone marrow (2). Treatment of mice with endotoxin (ET) produces an increase in the plasma concentration (3,4) of a factor(s) (colony stimulating factor [CSF]) capable of stimulating colony formation in agar cultures from progenitor cells of granulocytes and macrophages (GM-CFC). Injection of ET into mice after treatment with 5-FU accelerates the recovery of bone marrow CFU-S and of blood granulocytes (5).

This paper reports experiments which show that ET, administered after 5-FU injections, stimulates the recovery not only of CFU-S but also of GM-CFC (especially of a class of progenitors which give rise to large colonies), of erythropoiesis, and of platelet production. Furthermore, it is shown that a single injection of ET-free extracts prepared from pregnant mouse uterus containing CSF produces effects similar to those produced by ET.

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MATERIALS AND METHODS

Three-month-old male mice [C57BL, BALB/c, and (BALB/c × C57BL)F_1] were used. The animals were maintained in an air-conditioned animal house on a 12-hour day/night schedule, and fed a standard diet in cubes (Barastoc, Melbourne). The mouse strains used originally came from the Jackson Laboratories, Bar Harbor, ME, and were maintained by T. R. Bradley at the University of Melbourne and then in the animal house of The Cancer Institute (Melbourne). 5-FU (Roche Laboratories, Nutley, NJ) (6-mg/ml solution in pyrogen-free saline) was injected iv. ET (*Salmonella typhosa*) (090 1 lipopolysaccharide, Difco batch 3124-25) was used; it was dissolved in pyrogen-free 0.9% NaCl. Killed *Corynebacterium parvum* (7 mg/ml; Coparvax) was a gift from Wellcome Laboratories (Beckenham, Kent, UK). The pregnant mouse uterus extracts were prepared by the method of Bradley et al (6) and were shown to be ET-free using the actinomycin D (Merck and Co, West Point, PA) lethality assay of Pieroni et al (7) (table 1). The CSF activity of

TABLE 1.—Effect of combined ip injections of actinomycin D, ET, or tissue extracts on survival of mice at 2 days

Treatment*	No. of survivors at 2 days/ No. treated (%)
Actinomycin D, 18 μg	24/24 (100)
Actinomycin D, 18 μg, + ET, 0.1 μg	0/10
Actinomycin D, 18 μg, + ET, 0.01 μg	1/15 (7)
Actinomycin D, 18 μg, + ET, 0.001 μg	9/25 (36)
Actinomycin D, 18 μg, + C57BL mouse PU, 0.1 ml	10/10 (100)
Actinomycin D, 18 μg, + Swiss mouse PMUE(34), 0.1 ml	5/5 (100)
Actinomycin D, 18 μg, + BALB/c mouse PU, 0.1 ml	8/8 (100)

*PU = pregnant mouse uterus; PMUE(34) = placenta, membranes, uterus, and embryo extract No. 34.

the extracts was assayed by comparing their effects with those of dilutions of a laboratory reference, PMUE(33),[3] using the agar colony technique of Bradley and Metcalf (8) as modified by Bradley et al (9). An arbitrary unit of activity is defined as that contained in 0.4 μl of solution of preparation PMUE(33). To give an idea of its activity, 1 unit, when added to 10^4 bone marrow cells from normal mice, will give rise to about half the maximum number of colonies obtainable after 14 days of incubation. The laboratory reference has been shown to be stable when kept at –20°C for at least 1 year. In the assay of GM-CFC of cell suspensions, colonies were scored at 14 days and were classified in two broad groups (small, < 0.25 mm in diameter, and large, > 0.25 mm in diameter) on the basis of a preliminary study of the size distribution of the colonies. At least three replicate dishes were plated per sample; however, when GM-CFC content was low, as many as 30 dishes were plated per suspension, rather than increasing the total cells plated per dish. Liquid cultures of bone marrow (10) were carried out by placing 5×10^4 marrow cells in 1 ml of α-medium gassed with 5% O_2, 10% CO_2 in Falcon tubes for a period of 6 days with and without 0.1 ml of PMUE(33). At 5 days ^{125}I-IUdR was added, and the incubation was carried for a further day. The sample was prepared for counting as described previously (11).

The CFU-S content of bone marrow cell suspensions was assayed using the spleen colony assay of Till and McCulloch (12), injecting aliquots of bone marrow suspensions into ten isogeneic irradiated recipients [700 rads for BALB/c, 800 rads for C57BL, and 850 rads for (BALB/c × C57BL)F_1 mice]. The irradiation conditions have been described previously (13). Colonies were counted on spleens removed 10 days after irradiation and fixed in formol-acetic acid-ethanol. Counts were made using a binocular dissection microscope at 20 × magnification.

Platelet counts were done using a phase contrast microscope on blood samples dissolved in 1% ammonium oxalate (20 μl/ml). Wbcs were counted using a Coulter Counter model B.

Erythropoiesis was measured using ^{59}Fe. ^{59}Fe citrate (0.5 microcurie; 10 microcuries/μg) (Radiochemical Centre, Amersham, UK) was injected at different times after 5-FU and the mice were bled 3 days later. The radioactivity in the washed rbcs from 50 μl of blood was counted in a Packard Autogamma Counter model 522.

In all experiments, ten mice were used per treatment group.

RESULTS

Recovery of CFU-S After 5-FU Injections

Injection of 150 mg/kg of 5-FU into (BALB/c × C57BL)F_1 male mice (fig 1) decreased the CFU-S content of the marrow to a minimum of about 0.1% of normal at 2 days, after which recovery began and near-normal levels were reached by Day 7. In the mice that received ET after 5-FU, as Reissmann et al (5) have previously reported, recovery started earlier and CFU-S reached higher than normal numbers by Day 6. The doubling time for CFU-S in 5-FU-injected mice, with and without ET treatment, was about 12 hours.

Recovery of GM-CFC After 5-FU Injections

The behavior of the two types of operationally defined GM progenitors (GM-CFC), those capable of forming large colonies (GM-CFC-L) and those capable of forming small colonies (GM-CFC-S) in agar, was quite different after 5-FU treatment and ET injection (fig 2). The GM-CFC-L, like the CFU-S, reached a nadir at Day 2 in both 5-FU- and 5-FU + ET-treated mice. However, the 5-FU + ET-treated mice showed significantly lower values 1 day after 5-FU than did the 5-FU-treated mice. Recovery of GM-CFC-L was significantly faster in the 5-FU + ET-treated mice than in the 5-FU-treated mice (doubling time = 0.4 day vs 0.94 day) and reached supranormal levels by Day 7. At this time, femora from 5-FU + ET-treated mice contained 30 times more GM-CFC-L than femora from mice treated with 5-FU alone.

The GM-CFC-S diminished more slowly than the GM-CFC-L after 5-FU and 5-FU + ET treatment; they reached a nadir at Day 3 and recovered at the same rate in both 5-FU and 5-FU + ET-treated mice up to Day 5, when recovery slowed in the 5-FU-treated mice but continued at a fast rate in the 5-FU + ET-treated mice, so

[3]PMUE(33) = placenta, membranes, uterus, and embryo extract No. 33.

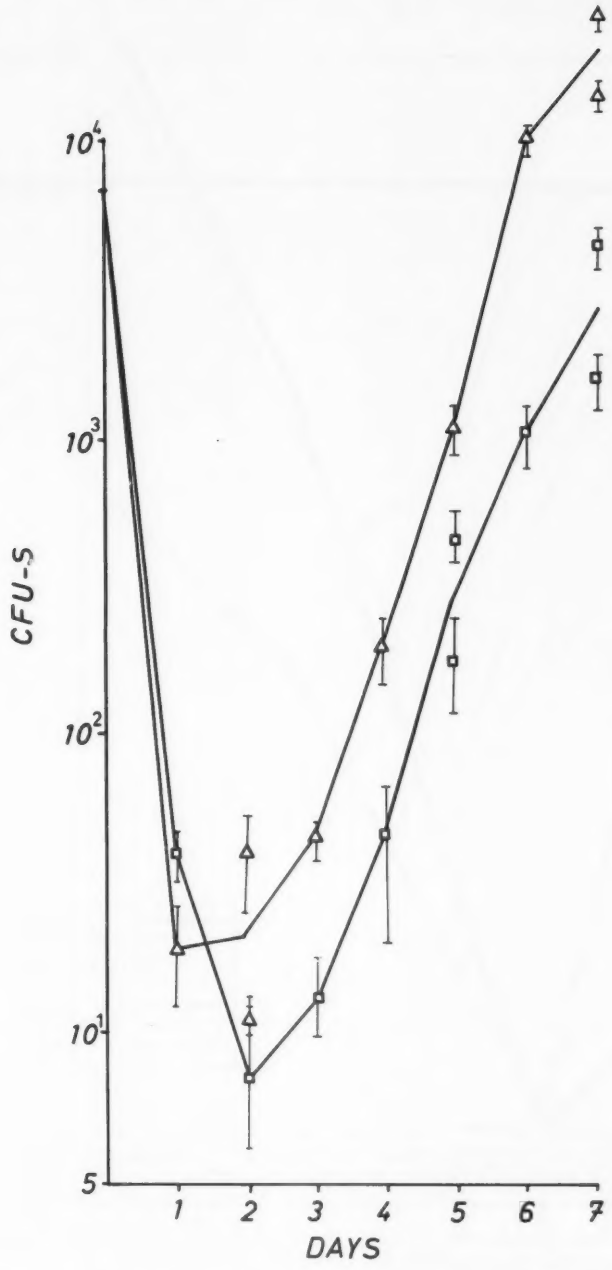

FIGURE 1.—Ordinate log scale. Mean (±SE) CFU-S content of femora of (BALB/c × C57BL)F_1 male mice treated with 5-FU (□ — □) or 5-FU + ET (△ — △). Each point represents the assay of CFU-S content of a pool of marrow obtained from 10 mice and injected into 10 irradiated recipients.

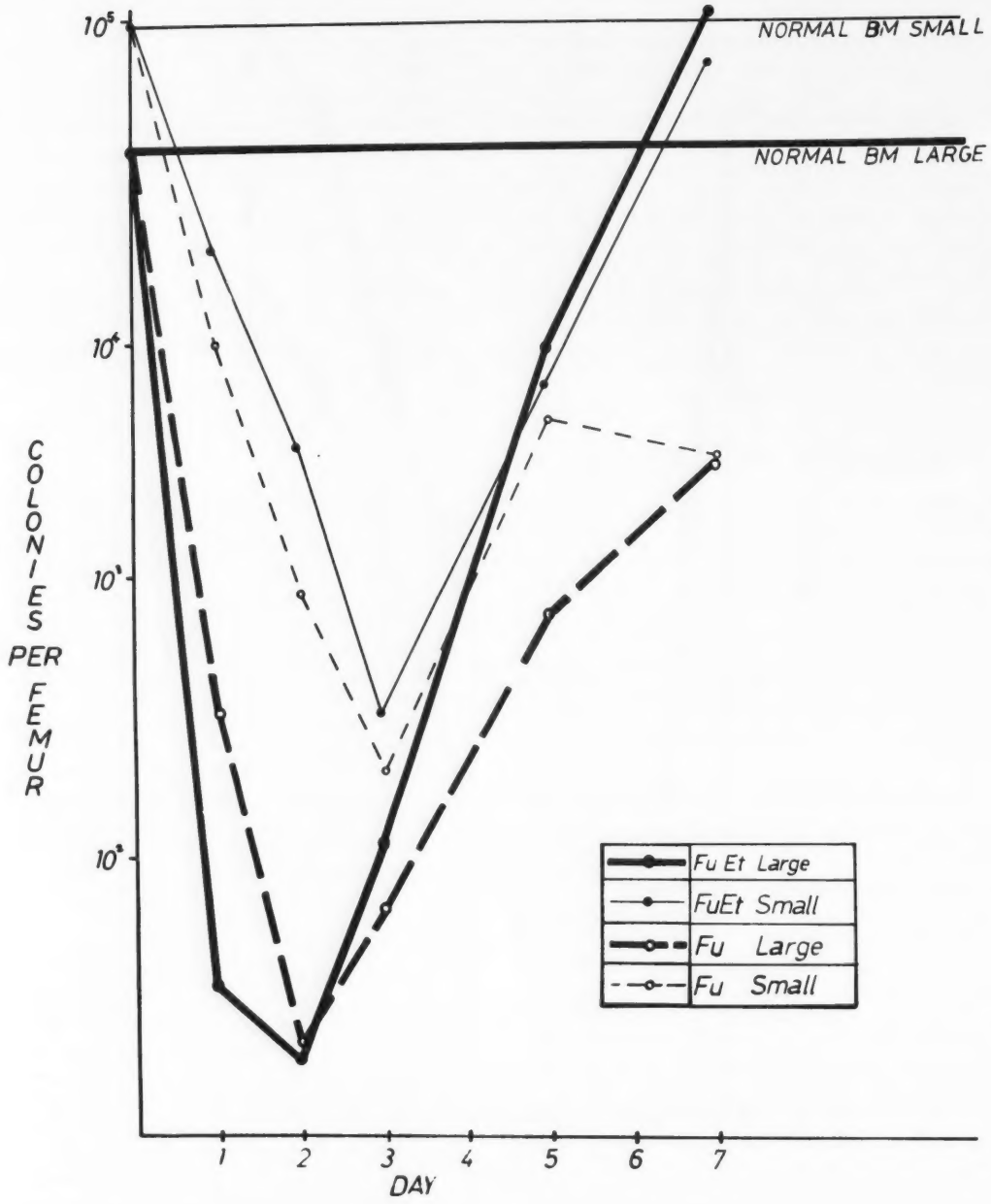

FIGURE 2.—Ordinate log scale. Regeneration of GM-CFC after 5-FU and ET treatment. The points shown are averages obtained from groups of 10 mice. Where CFC content was low, 10^5 cells were plated on each of 30 dishes. The reproducibility of colony counts was such that the standard error was < 0.1 of the mean. Calculation of doubling times (DT) by linear regression analysis of log GM-CFC-L vs time for this and another similar experiment gave the following values: DT for 5-FU, 0.77 ± 0.05 and 1.1 ± 0.10 day; DT for 5-FU + ET, 0.38 ± 0.02 and 0.41 ± 0.02 day.

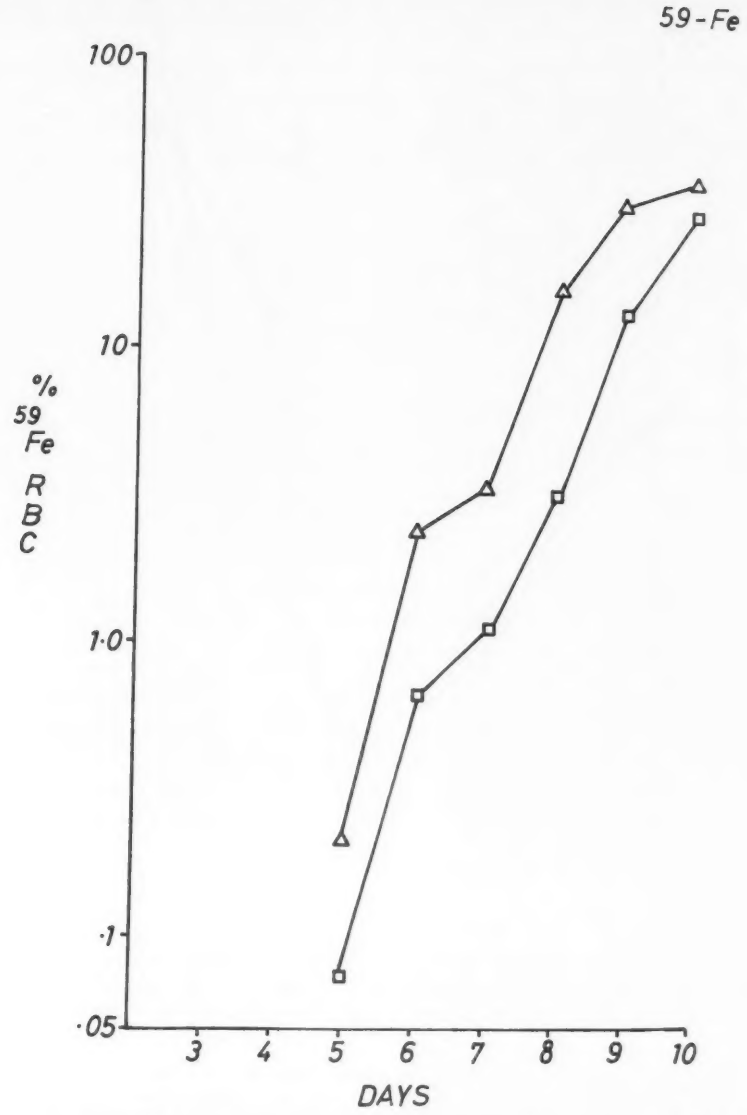

FIGURE 3.—Ordinate log scale. 72-hr incorporation of ^{59}Fe into rbcs from 1 ml of blood from (BALB/c × C57BL)F_1 male mice injected with 5-FU (□ — □) or 5-FU + ET (△ — △). The points shown are averages from groups of 10 mice. Standard errors (not shown) were < 0.2 of the mean.

that there was a marked difference between the two groups by Day 7.

Recovery of Erythropoiesis After 5-FU Injections

There was no measurable incorporation of ^{59}Fe in rbcs when tracer was injected 3 and 4 days after 5-FU (fig 3). Recovery started about 1 day earlier in 5-FU + ET-treated mice than in the 5-FU-treated mice, but once started, recovery continued at a similar rate in both groups. The recovery curve showed two rates, an initial rapid one with a doubling time of ~0.35 day and a slower one with a doubling time of 0.7 day. This type of recovery of erythropoiesis is seen in lethally irradiated mice injected with bone marrow and probably follows the same kinetic rules (14).

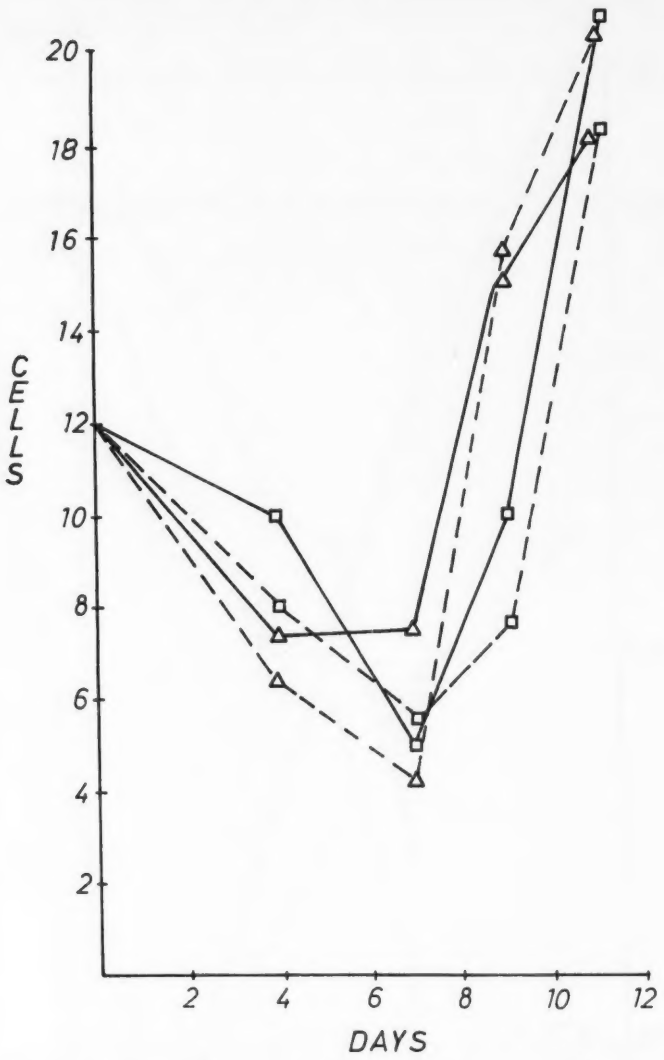

FIGURE 4.—Ordinate linear scale. Platelet counts of (BALB/c × C57BL)F_1 mice treated with 5-FU (□ — □) or 5-FU + ET (△—△). Leukocyte counts of (BALB/c × C57BL)F_1 mice treated with 5-FU (□ - - - □) or 5-FU + ET (△ - - - △). The points shown are averages from groups of 10 mice. Ordinate cell counts × 10^{-5} for platelets and × 10^{-3} for leukocytes. Standard errors (not shown) were < 0.1 of the mean.

Recovery of Wbc and Platelet Counts After 5-FU Injections

Wbc counts decreased to their nadirs 1 week after injection of 150 mg/kg of 5-FU (fig 4). ET injections, as Reissmann et al (5) have previously reported, produced a more prompt recovery of wbc counts. Differential counts of peripheral blood smears showed that granulocytes appeared (2% for 5-FU treatment; 8% for 5-FU + ET treatment) on Day 9 and markedly increased on Day 11 (20% for 5-FU treatment; 37% for 5-FU + ET treatment). The overshoot in wbc counts was similar in both groups.

Platelet counts also decreased after 5-FU, reaching a nadir at 1 week. The platelet counts at 4 days were lower in mice that received ET. Recovery of platelet counts

TABLE 2.—Effect of a single ip injection of ET (6 μg) and 0.2 ml of *C. parvum* ip (7 mg/ml) 4 hrs after 150 mg/kg of 5-FU on GM-CFC and CFU-S content of marrow and spleen of C57BL male mice 7 days after injection of 5-FU

	Mean ± SE			
	5-FU	5-FU + ET	5-FU + *C. parvum*	Normal
Cells/femur × 10^{-6}	4.2	7.3	4.8	36.0
GM-CFC-L × 10^{-3}	1.2 ± 0.2	43.0 ± 0.2	22.0 ± 3.0	60.0 ± 4.0
GM-CFC-S × 10^{-3}	1.4 ± 0.3	22.0 ± 4.0	16.0 ± 4.0	160.0 ± 4.0
Bone marrow CFU-S × 10^{-3}	5.2 ± 0.3	22.0 ± 1.4	18.0 ± 2.7	ND*
Spleen GM-CFC-L × 10^{-3}	2.0 ± 2.0	1.0 ± 0.5	8.0 ± 2.0	5
Spleen GM-CFC-S × 10^{-3}	0.4 ± 0.4	1.0 ± 0.5	28.0 ± 16.0	5

*ND = not determined.

TABLE 3.—Effect of a single ip injection of 1 ml (2500 CSF units equivalent) of an extract of isogeneic pregnant mouse uterus (PU) and of an extract of allogeneic (Swiss mouse) pregnant mouse placenta, membranes, uterus, and embryo (PMUE[34]) 4 hrs after 150 mg/kg of 5-FU on bone marrow and spleen GM-CFC content 7 days after 5-FU in C57BL male mice

	Mean ± SE		
	5-FU	5-FU + PU	5-FU + allogeneic PMUE(34)
Cells/femur × 10^{-6}	3.9 ± 0.3	7.2 ± 0.3	6.4 ± 0.3
GM-CFC-L × 10^{-3}	1.8 ± 1.6	16.0 ± 3.0	18.0 ± 3.0
GM-CFC-S × 10^{-3}	2.8 ± 0.4	23.0 ± 5.0	19.0 ± 4.2
Cells/spleen × 10^{-6}	114.0	98.0	126.0
GM-CFC-L × 10^{-3}	0	0	0.65
CM-CFC-S × 10^{-3}	0	0.3	5.2

started on Day 7 and ET-injected mice showed 50% higher counts on Day 9 compared to mice that received 5-FU only. The overshoot in platelet counts at Day 11 was similar in both groups.

Effect of *C. parvum* on Hemopoietic Recovery After 5-FU Injections

The hemopoietic recovery on Day 7 after 5-FU treatment (table 2) was measured in C57BL male mice injected 4 hours after 5-FU with 0.2 ml (1.4 mg) of killed *C. parvum* suspension and was compared to the effects of 6 μg of *S. typhosa* ET. As with ET, *C. parvum* injection increased the marrow content of CFU-S and GM-CFC (both large and small), although to a smaller degree in the case of GM-CFC-L. Spleen GM-CFC content was increased more by *C. parvum* than by ET, but was negligible in relation to total bone marrow content.

Effects of CSF-Containing Extracts on Recovery After 5-FU Injections

Single injections of 1 ml of ET-free extracts of C57BL pregnant mouse uteri (6) containing large quantities of CSF (1 ml = 2500 arbitrary units) were assayed for their effect on C57BL and BALB/c mice 7 days after 5-FU (tables 3 and 4). C57BL pregnant mouse uterus significantly increased both classes of bone marrow GM-CFC and had no effect on spleen GM-CFC. PMUE(34) prepared from an allogeneic source (Swiss mice) produced similar increases in bone marrow GM-CFC and CFU-S and also increased spleen GM-CFC (table 3).

A pregnant mouse uterus extract prepared from BALB/c mice (table 4) produced an increase in BALB/c marrow CFU-S and GM-CFC similar to that produced by 6 μg of ET. Bone marrow cells (5×10^4) from mice treated with 5-FU and either ET or pregnant mouse

TABLE 4.—Effects of a single ip injection of 6 μg of ET and 1 ml of an extract of isogeneic pregnant mouse uterus (PU) 4 hrs after 150 mg/kg iv of 5-FU on GM-CFC, CFU-S content, and ^{125}I-IUdR uptake by bone marrow of BALB/c male mice 7 days after 5-FU

	Mean ± SE			
	5-FU	5-FU + ET	5-FU + PU	Normal
Cells/femur × 10^{-6}	2.4	4.0	4.6	20.4
GM-CFC-L × 10^{-3}	0.7	5.0	6.0	26.0
GM-CFC-S × 10^{-3}	2.0	18.0	21.0	68.0
^{125}I-IUdR uptake on Day 6 in culture (cpm × 10^{-5})	1.0 ± 0.08	5.0 ± 0.3	4.8 ± 0.2	2.3 ± 0.2
CFU-S/femur × 10^{-3}	0.42 ± 0.06	2.8 ± 0.2	3.1 ± 0.2	5.2 ± 0.4

uterus extract, grown in vitro in liquid culture for 6 days in the presence of a colony-stimulating factor, showed an incorporation of ^{125}I-IUdR into DNA fivefold higher than that of 5-FU marrow and twice that of normal marrow.

DISCUSSION

A single injection of ET given 4 hours after 150 mg/kg of 5-FU stimulated recovery of CFU-S, GM-CFC, and erythropoiesis. Recovery of marrow megakaryocytes, which showed a striking repopulation after the initial depopulation by 5-FU, was brought forward by about 1 day by ET injection (15). This is also the picture for CFU-S and erythropoiesis. However, in the case of the granulocyte macrophage progenitor cells which were capable of forming large colonies in agar, ET did not hasten the onset of recovery but rather increased the rate of recovery (fig 2). Recovery of CFU-S and GM-CFC was also stimulated by a single injection of extracts of pregnant mouse uterus containing CSF (6). GM-CFC-L are a class of granulocyte macrophage progenitor cells capable of producing an average of 5000 cells each in culture (Bradley TR, unpublished data). The fact that animals treated with 5-FU and ET had 10^5 cells/femur of these progenitor cells means that the whole marrow (≃20 × femur) had 2×10^6 progenitors and thus the potential of producing about 10^{10} terminal differentiated cells. Mice not treated with 5-FU have 40 times fewer of these cells.

An hypothesis which would account for the observed effects is that ET stimulates the production of, and pregnant mouse uterus extracts contain, GM-CSF and a factor(s) which stimulates the early onset of CFU-S production, and consequently of a CFU-S-led recovery of GM-CFC, erythropoiesis, and platelet production. The fact that recovery of erythropoiesis is stimulated by ET supports the concept of a CFU-S-led recovery, for ET is known to depress ongoing erythropoiesis and the response of ex-hypoxic mice to erythropoietin (16). Thus, stimulation by ET must reflect action at an earlier stage than the erythropoietic responsive cell. Increases in GM-CSF plasma levels could account for the more rapid regeneration and greater amplification in the GM-CFC compartment. The increase in GM-CSF 6 hours after ET injection into 5-FU-treated mice was similar to that observed in normal mice, while no increase was observed in mice treated with 5-FU only (Bradley TR, and Hodgson GS, unpublished data). The earlier decrease in GM-CFC-L in 5-FU + ET-treated mice may also play a part in stimulating CFU-S regeneration in view of the observations of Lajtha et al (17) on the production of inhibitory and stimulatory factors in the marrow. The importance of the high CFU-S- and GM-CFC-generating capacity of the CFU-S that survive 5-FU (2) in determining the response to ET must be evaluated. It is apparent that insufficient stimuli arise in the 5-FU-treated mouse to unmask the full potential of these high self-renewal capacity stem cells. Factors which are either generated by ET or are contained in the tissue extracts injected into the mice seem able to accomplish earlier triggering of CFU-S regeneration and a fuller expression of their potential to generate hemopoietic progenitor cells.

One aspect of the response to ET and *C. parvum* of the 5-FU-treated animal was the minor contribution of the spleen, in contrast to what is observed in normal animals which show a great increase of the spleen GM-CFC pool (18) after *C. parvum*. It may well be that in normal mice there is little room for expansion in bone marrow so that "spill over" to the spleen occurs.

The significance of the present observations to the field of cancer chemotherapy is clear. It now appears feasible to stimulate hemopoietic recovery using factors which are produced by the organism in response to bacterial products, rather than using bacterial products themselves. Of special relevance is the striking increase observed in the progenitors of granulocytes and macrophages, which play a central role in general immunity (19) and in resistance against tumors (20). The changes in T- and B-cell populations after the stimulating treatment given after 5-FU have yet to be evaluated.

Much work must be done in characterizing the factor(s)

present in pregnant mouse uteri, which contain high levels of not only GM-CSF, but probably also other hemopoietic growth stimulators. Human CSF is produced by human placentas in culture (21), and thus a start has been made in preparing factors suitable for similar studies of human hemopoiesis.

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Acute Cardiovascular Alterations Induced by Low Doses of Adriamycin, Rubidazone, and Daunorubicin in the Anesthetized Beagle Dog [1,2,3]

Eugene H. Herman* and Robert S. K. Young [4]

SUMMARY

Iv injections of Adriamycin (0.375–3.0 mg/kg), rubidazone (0.75–6.0 mg/kg), or daunorubicin (0.75–6.0 mg/kg) given within 1 minute produced immediate hypotension in anesthetized beagle dogs. The threshold hypotensive dose for Adriamycin (0.375 mg/kg, 7.5 mg/m^2) was lower than that for either rubidazone (0.75 mg/kg) or daunorubicin (1.5 mg/kg). Recovery from hypotension was more rapid after administration of rubidazone or daunorubicin than after Adriamycin. Adriamycin (1.5–3.0 mg/kg) caused respiratory distress and depressed mean arterial pressure an average of 54%–82% for up to 30 minutes. The hypotension was almost eliminated when the Adriamycin (1.5 mg/kg) infusion period was extended to 15 minutes. Significant increases in plasma histamine concentrations, coinciding with the peak hypotensive response, were detected within 1 minute after infusion of either Adriamycin (1.5 mg/kg) or rubidazone (1.5 mg/kg). Similar effects were noted after 6.0 mg/kg of daunorubicin. It appears that the acute cardiovascular effects of the three anthracycline compounds are mediated through release of histamine.

[Cancer Treat Rep 63:1771–1779, 1979]

Adriamycin (ADR), an anthracycline antibiotic, is one of the most useful agents currently utilized in cancer chemotherapy (1,2). One of the major concerns during clinical usage is the development of a chronic cardiomyopathy leading to pump failure (3,4). Recent clinical observations indicate that ADR is also capable of initiating rapid changes in cardiovascular function; this activity appears characteristic of an allergic reaction in that it may occur shortly after ADR administration and can be either localized or systemic (5). In beagle dogs, small doses of ADR have been shown to cause a precipitous fall in arterial blood pressure (6). The hypotension occurs simultaneously with an increase in serum histamine concentration and can be attenuated by agents modifying the action of histamine (6). Other anthracycline antineoplastic agents such as rubidazone and daunorubicin (fig 1) are also being used clinically. The present studies were initiated to determine whether rubidazone and daunorubicin could also elicit immediate decreases in systemic arterial blood pressure and whether extending the duration of the infusion period would alter ADR-induced hypotension. The effects of the three anthracycline compounds on plasma histamine levels were also studied.

MATERIALS AND METHODS

Adult beagle dogs of either sex, weighing 8.0–11.0 kg, were utilized in all experiments.

Experimental preparation.—Each animal was anesthetized with 30 mg/kg of pentobarbital sodium (iv). A tracheal cannula was inserted and the left femoral artery and vein were isolated and cannulated with polyethylene tubing. The arterial cannula was connected to a pressure transducer to monitor systolic, diastolic, and mean ar-

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[2]Presented in part at the 69th annual meeting of the American Association for Cancer Research, Washington, DC, Apr 5–8, 1978.

[3]Adriamycin, daunorubicin, and rubidazone were obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

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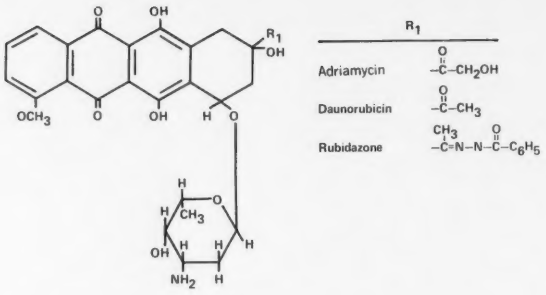

FIGURE 1.—Structures of ADR, rubidazone, and daunorubicin.

terial pressure while the venous cannula was utilized for drug injections. Lead II of the electrocardiogram was recorded through subcutaneous limb electrodes and the heart rate was determined from the electrocardiogram through a Hewlett-Packard 350-3400A Cardiotach preamplifier. All physiologic functions were recorded on a Hewlett-Packard polygraph. Each animal was allowed to stabilize for 15–30 minutes before drug injection. The clinical formulation of each anthracycline was diluted to a dose volume of 5 mg/ml with 0.9% physiologic saline (ADR and daunorubicin) or 5 mg/4 ml of solvent consisting of 37.2 mg of glycine and 0.36 of sodium glycinate/ml of water (rubidazone).

Anthracycline administration.—Doses of the three anthracyclines selected for comparison were based on the range of hypotensive responses found in previous studies with ADR (6). Four doses of each agent were given to eight dogs to determine the effects on the systemic arterial pressure. The four test doses were divided so that each animal received only two, a low dose followed after 30 minutes by a second higher dose. Each two-dose regimen was repeated in four separate experiments. All doses were injected by rapid manual infusion within 1 minute according to the following sequence: *ADR*—four dogs each received 0.375 (7.5 mg/m^2) and 1.5 mg/kg or 0.75 and 3.0 mg/kg; *rubidazone*—four dogs each received 0.75 and 3.0 mg/kg or 1.5 and 6.0 mg/kg; *daunorubicin*—four dogs each received 0.75 and 3.0 mg/kg or 1.5 and 6.0 mg/kg. Control values were measured just before anthracycline administration. The actual changes in mean arterial pressure and heart rate were determined 1, 5, 15, and 30 minutes after each injection. The readings obtained were converted to percent of control values from which calculations of mean and standard error (SE) were made at each time interval.

Varying ADR infusion rates.—The influence on systemic arterial pressure of varying ADR infusion rates was studied in 12 dogs. ADR (1.5 mg/kg) was administered either in < 1 minute or over a 4- or 15-minute period. Each infusion rate was repeated in four separate experiments. Experiments were terminated 30 minutes after completion of the infusion. Control values were obtained before ADR administration and the actual observed changes were followed and recorded as described above.

Histamine determinations.—A total of 12 dogs were given a single rapid iv bolus injection of 1.5 mg/kg of ADR, rubidazone, or daunorubicin (four animals each). In three additional dogs the dose of daunorubicin was increased to 6.0 mg/kg. Blood samples (7 ml) were collected through the femoral artery cannula before and at intervals of 1 and 15 minutes after anthracycline administration. Plasma histamine determinations were made according to the method of Shore et al (7), modified for use in the Technicon AutoAnalyzer.

RESULTS

Acute Anthracycline Effects

ADR produced a rapid decrease in arterial pressure. The fall began within 20–30 seconds and was maximal 1 minute after injection. Control values for mean arterial blood pressure and heart rate in these animals were 155 ± 6 mm Hg and 189 ± 10 beats/minute respectively. The observed changes in mean arterial pressure and heart rate during the 30-minute observation period after increasing doses of ADR are summarized in figure 2.

The magnitude of the hypotensive response varied from 6% (–10 ± 2 mm Hg) after the lowest dose (0.375 mg/kg) to 72% (–92 ± 8 mm Hg) after the highest dose (3.0 mg/kg). At the lower doses (0.375 and 0.75 mg/kg), the mean arterial pressure returned to control levels within 5–15 minutes, whereas after the two higher doses, recovery was not complete even at the end of the 30-minute observation period. During the hypotensive period, moderate changes in heart rate occurred. An initial tachycardia (+23 ± 7 beats/minute) occurred after injection of 0.75 mg/kg of ADR, whereas a 9%–22% (–29 ± 11 beats/minute) decrease in rate occurred within 5 minutes after the higher doses. The amplitudes of the R and T waves were reduced during the peak of the hypotensive response.

The respiratory pattern was modified by the 1.5- and 3.0-mg/kg doses of ADR. The alterations observed in most animals varied from a labored, shallow, rapid rate to complete cessation. When apnea did occur, animals were maintained by several minutes of artificial respiration.

Rubidazone exerted little hypotensive activity at a dose below 1.5 mg/kg (fig 3). At the 1.5-mg/kg dose a certain variability was noted in the group: the mean arterial pressure declined 48%–50% (–65 to 70 mm Hg) in two animals but only 13%–17% (–20 to 25 mm Hg) in the other two animals. The magnitude of the hypotensive responses seen after all doses of rubidazone is summarized in figure 4. In these animals control values for mean

arterial blood pressure and heart rate were found to be 165 ± 18 mm Hg and 169 ± 17 beats/minute respectively.

Respiratory depression as described above after ADR administration occurred in five of eight animals receiving the two higher rubidazone doses. A volume of solvent equivalent to a 6-mg/kg dose of rubidazone injected into one dog did not alter blood pressure or heart rate.

Daunorubicin doses of 0.75–3.0 mg/kg produced only minor and transient decreases in mean arterial pressure (−4 ± 2 to −10 ± 3 mm Hg) (fig 4). A moderate (38%) (−60 ± 21 mm Hg) reduction occurred after the 6.0-mg/kg dose. Recovery of arterial pressure after this dose was essentially complete within 15 minutes. Control mean arterial blood pressure and heart rate in this group

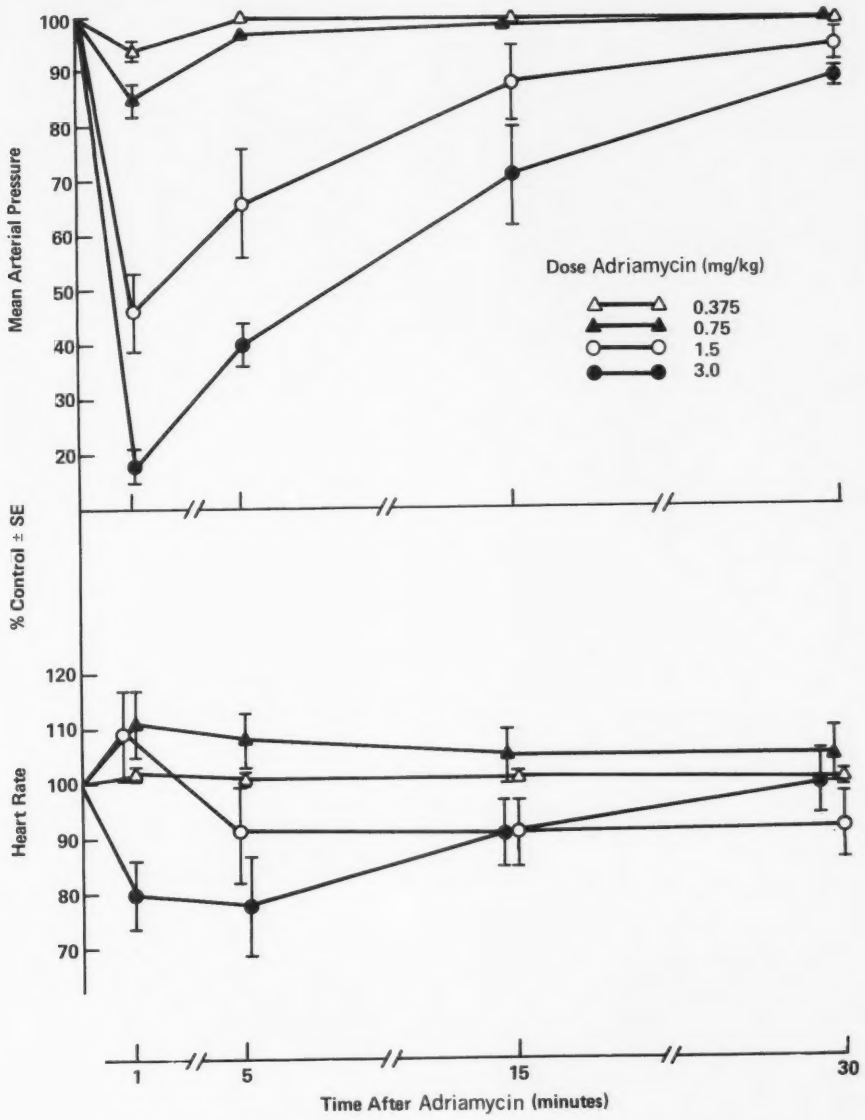

FIGURE 2.—Effect of rapid iv injection of 0.375, 0.75, 1.5, or 3.0 mg/kg of ADR on mean arterial pressure and heart rate in the anesthetized beagle dog. Each point represents the SEM from 4 separate experiments.

of animals were found to be 173 ± 16 mm Hg and 188 ± 5 beats/minute respectively.

Varying ADR Infusion Rates

Groups of four dogs were given iv infusions of ADR (1.5 mg/kg) in either < 1 minute or over a 4- or 15-minute infusion period. Administration of ADR in < 1 minute or over a 4-minute period resulted in identical decreases in mean arterial pressure (fig 5). The rates of recovery over the 30-minute experimental period were also identical. When the infusion period was extended to 15 minutes the fall in mean arterial pressure was essentially negated (fig 5). However, one of four dogs in this 15-minute infusion

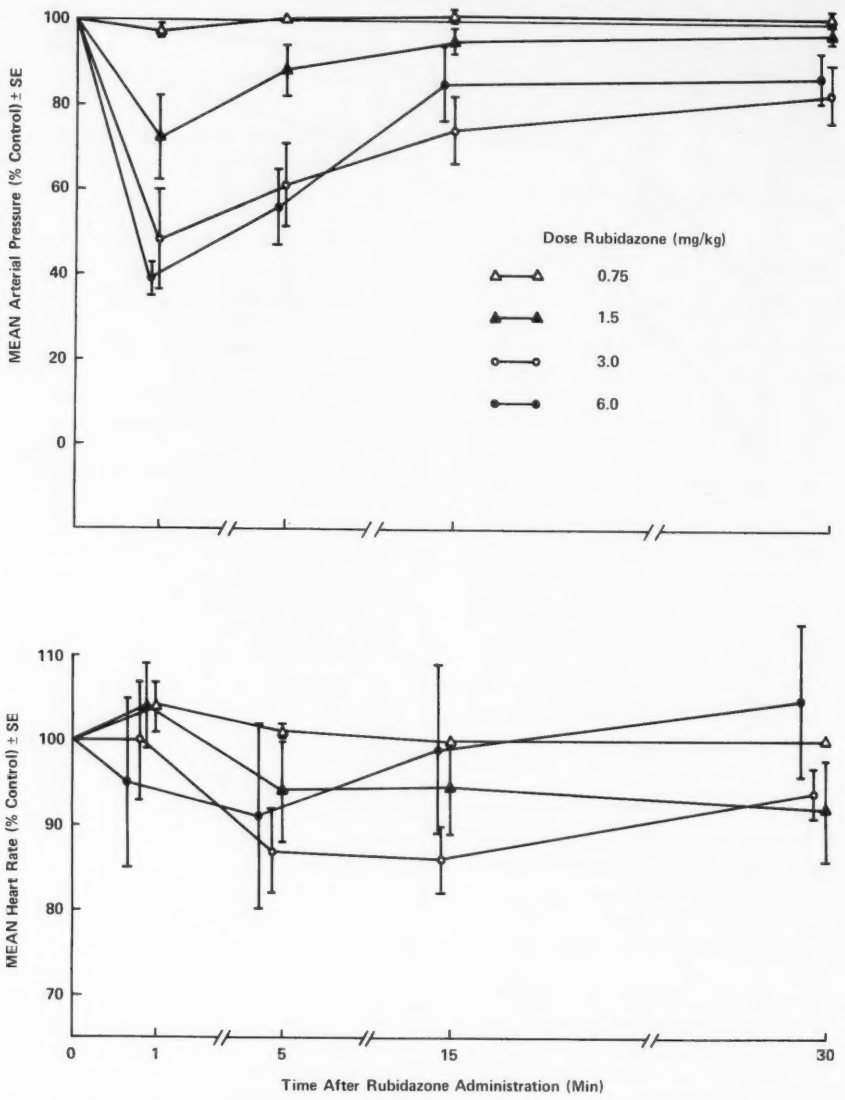

FIGURE 3.—Effect of rapid iv injection of 0.75, 1.5, 3.0, or 6.0 mg/kg of rubidazone on mean arterial pressure and heart rate in the anesthetized beagle dog. Each point represents the SEM from 4 separate experiments.

group developed allergic-like hives over a good portion of the body surface. This phenomenon was not noted in any other infusion group.

Histamine Determinations

Injection of 1.5 mg/kg of ADR or rubidazone caused significant decreases in mean arterial pressure (fig 6, top). The severity of this response was greater after ADR. Simultaneously with the peak hypotensive response each agent caused elevations in serum histamine concentrations (fig 6, bottom). The magnitudes of the initial increases were similar for both agents. However, after 15 minutes the serum histamine concentrations

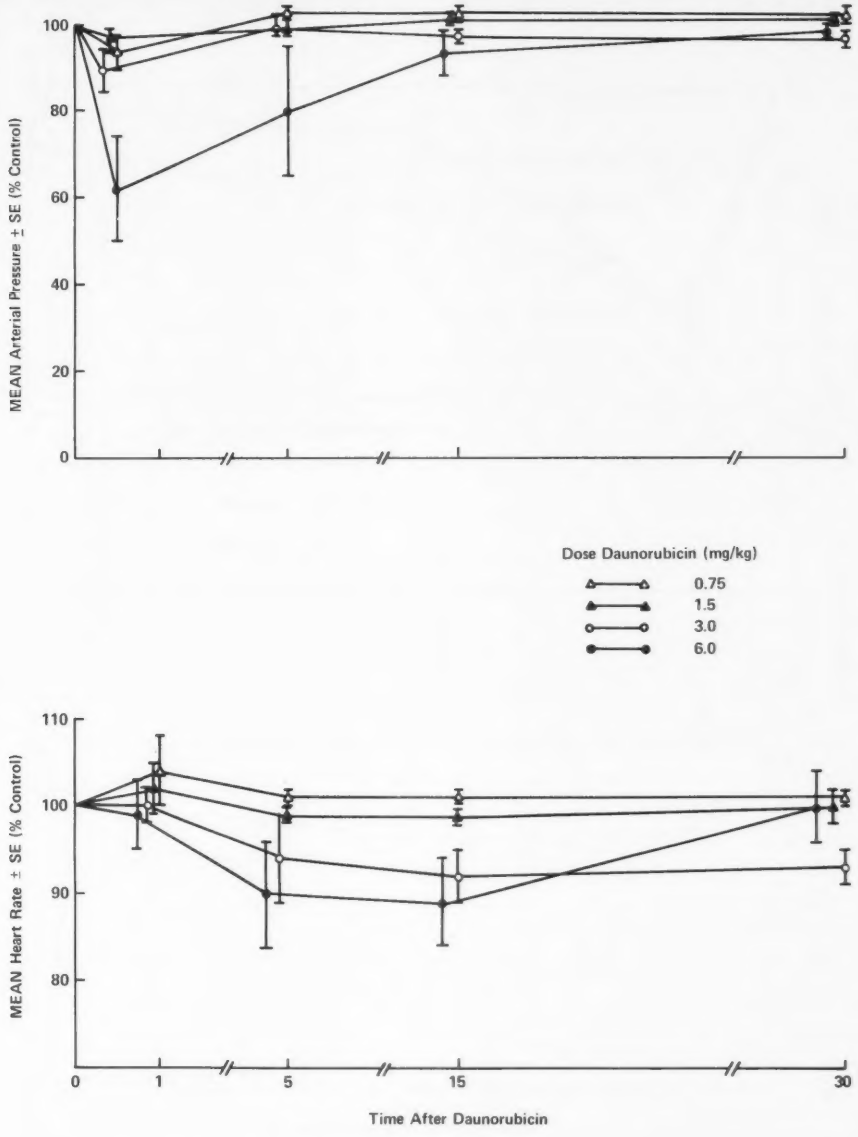

FIGURE 4.—Effect of rapid iv injection of 0.75, 1.5, 3.0, or 6.0 mg/kg of daunorubicin on mean arterial pressure and heart rate in the anesthetized beagle dog. Each point represents the SEM from 4 separate experiments.

were still significantly elevated in ADR-treated dogs but had essentially returned to control levels in rubidazone-treated animals. The same dose of daunorubicin (1.5 mg/kg) caused only minimal changes in both mean arterial pressure and plasma histamine concentrations. Significant increases in plasma histamine concentration (14 and 30 ng/ml) were noted in two of three dogs within 1 minute after a 6.0-mg/kg dose of daunorubicin. A modest 25%–35% decrease in mean arterial pressure occurred simultaneously. By 5 minutes the histamine concentration had almost returned to control levels in these two animals receiving the 6.0-mg/kg dose of daunorubicin.

DISCUSSION

The present study shows that iv administration of small doses of ADR to the anesthetized beagle dog evokes rapid changes in certain cardiovascular functions. As in pre-

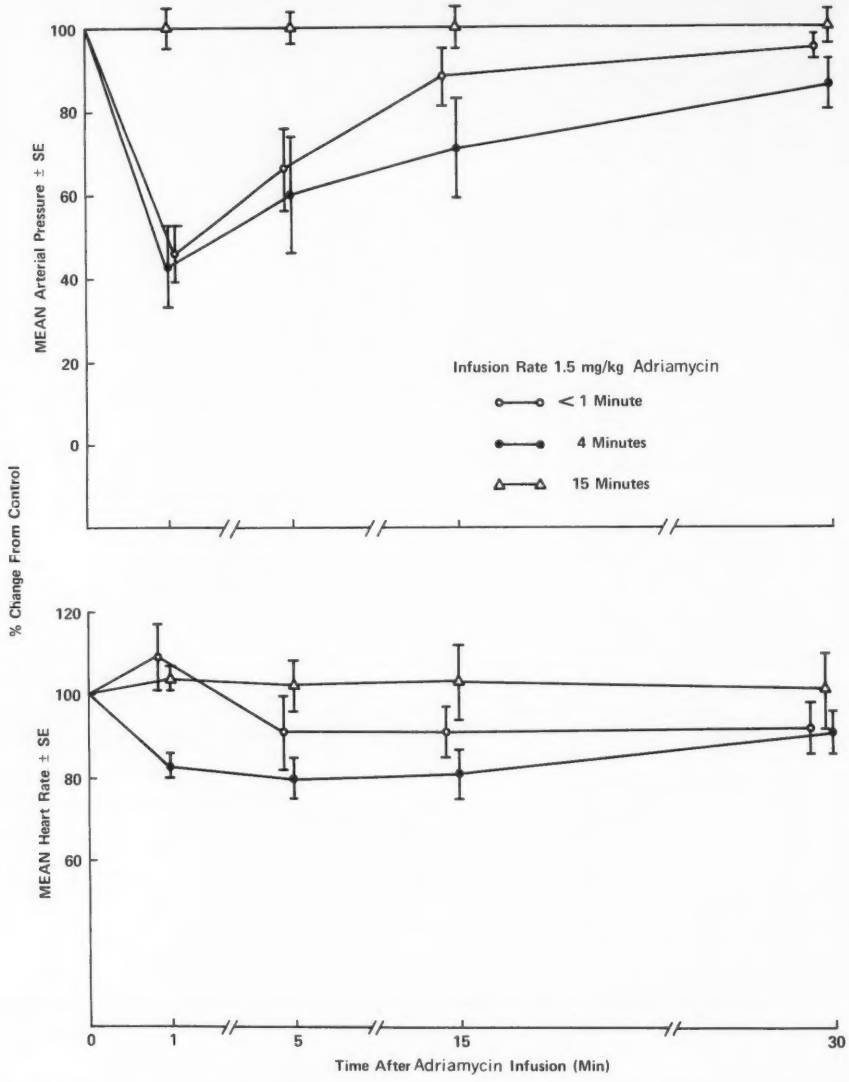

FIGURE 5.—Effect of a 1.5-mg/kg dose of ADR infused iv in either < 1 min or over a 4- or 15-min period on mean arterial pressure and heart rate in the anesthetized beagle dog. Each point represents the SEM from 4 separate experiments.

vious studies the most sensitive indicator of acute ADR activity was systemic arterial pressure, which was depressed by all doses of the agent tested (6). In the present experiments these actions occurred with other anthracyclines such as rubidazone and daunorubicin, although there were quantitative differences in the hypotension produced by these anthracyclines. ADR (0.375 mg/kg) caused a 6% decrease in mean arterial pressure, whereas 3.0 mg/kg of daunorubicin was required to produce a similar effect. The action produced by rubidazone, the ben-

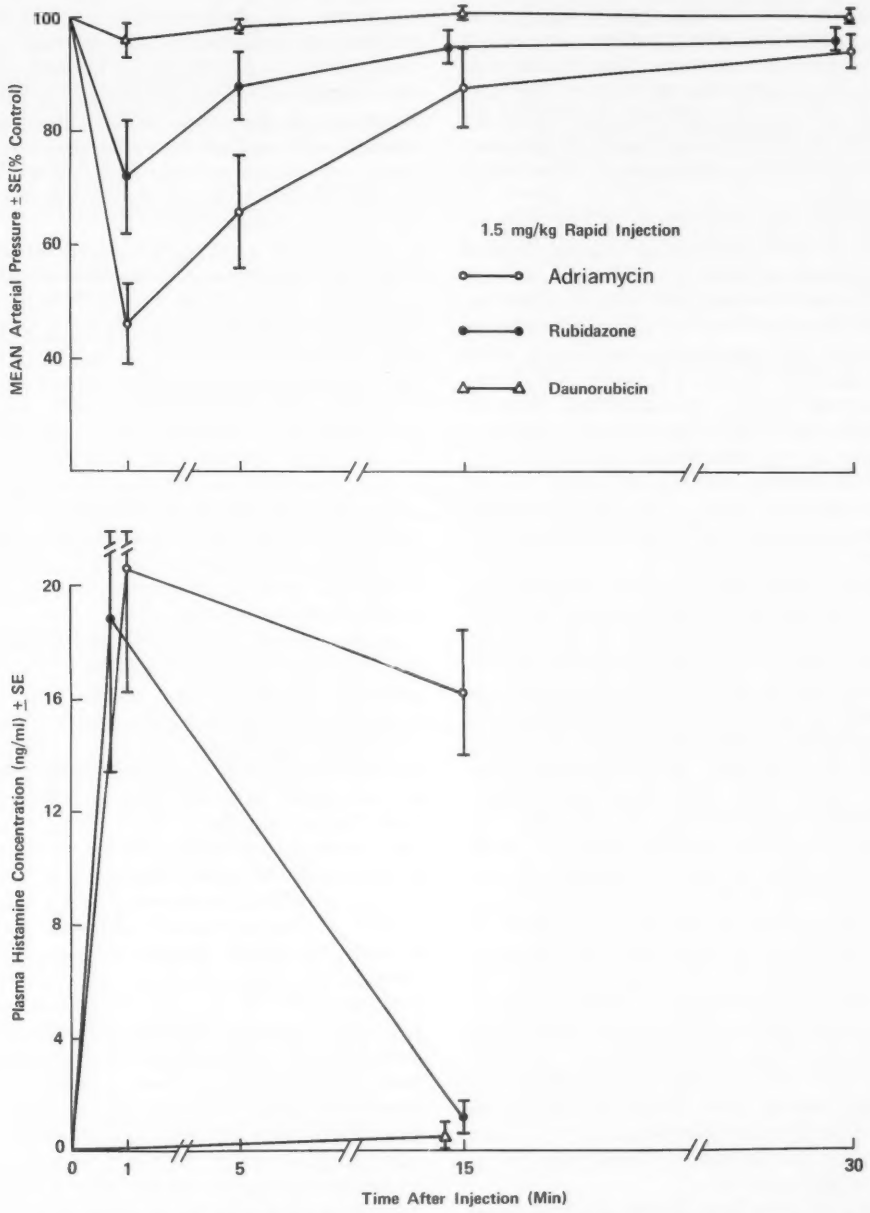

FIGURE 6.—Comparison of the change in mean arterial pressure and plasma histamine concentrations following rapid iv administration of 1.5 mg/kg of ADR, rubidazone, or daunorubicin. Each point represents the SEM from 4 separate determinations.

zoyl hydrazone derivative of daunorubicin, was intermediate between that of ADR and that of daunorubicin.

The decrease in blood pressure induced by each anthracycline agent was delayed approximately 15–30 seconds following administration. Such a latent period is not observed after the administration of substances such as acetylcholine or histamine, which produce an immediate relaxation of vascular smooth muscle. Thus, this suggests that the effects of anthracyclines are indirect. The most convincing evidence that the cardiovascular effects are indirect was the demonstration of significant increases in plasma histamine concentrations after ADR or rubidazone administration.

Comparable peak histamine concentrations were detected by 1 minute and corresponded with the maximal decline in blood pressure induced by 1.5-mg/kg doses of ADR or rubidazone. It seems likely that a critical anthracycline plasma concentration must be reached for this activity to occur. For example, the decrease in blood pressure was identical whether 1.5 mg/kg of ADR was infused in < 1 minute or over a 4-minute period. However, when the infusion period was extended over 15 minutes, with presumably a lower initial plasma concentration, the hypotensive response was markedly attenuated. We did not determine whether a similar reduction in response could be achieved if higher ADR doses were given over longer periods.

In spite of the similar peak histamine concentrations detected at the 1.5-mg/kg dose, ADR reduced systemic arterial blood pressure to a greater extent than rubidazone. A partial explanation for this discrepancy may be the observation that plasma histamine concentrations rapidly returned to control levels in rubidazone-treated animals but remained significantly elevated 15 minutes following ADR administration. At the 1.5-mg/kg dose, daunorubicin exerted only minor hypotensive activity and produced little change in plasma histamine. This variation in response might be related to differences in the structural characteristics of ADR, rubidazone, and daunorubicin. Modification of the molecule in the C-9 side chain causes differences in the membrane transport, intracellular accumulation, and physiologic handling of the anthracycline antibiotics (8). Daunorubicin, being the least polar and most lipid-soluble compound, is more readily bound to and taken up by the cell (8). Being more polar, ADR would perhaps be more disruptive to the cell membrane. There is evidence that slight modification of the tetracycline molecule alters histamine-releasing capability. In one in vitro study chlortetracycline produced significant rat mast cell disruption, whereas tetracycline itself was much less active (9).

Mast cells are the most likely source of the increased plasma levels of histamine detected after anthracycline administration. Several mechanisms which can ultimately lead to histamine release have been proposed from in vitro mast cell studies. Releasing agents such as concanavalin A or anti-IgE antibody appear to require an initial stimulating event at the cell surface (10,11). Responses of mast cells to these agents can be differentiated from those of compound 48/80 and the calcium ionophore A23187 by an initial increase in cyclic adenosine monophosphate, a requirement for phosphatidylserine in the medium, and a relatively slow release reaction (12). Compound 48/80 and A23187 release histamine more rapidly than concanavalin A and anti-IgE and probably alter different sites in the release process (12). It appears that, whatever the mechanism, cyclic adenosine monophosphate and calcium not only modulate but may also be directly involved in the biochemical processes responsible for histamine release (12).

At present it is not possible to put anthracycline histamine release into any of the above categories. A few studies in other systems may be relevant. Recently, mammalian erythrocytes were found to be more susceptible to hypotonic lysis after a 30-minute incubation with daunorubicin (13). The authors suggest that the effect may be due to a membrane action of the drug. ADR has been reported to inhibit Na+-K+ dependent adenosine triphosphatase activity (14). Inhibition of this enzyme system, located in the cell membrane, might initiate a series of events leading to histamine release. Finally, anthracyclines are also chelating agents (15) and could alter a critical membrane or cellular cation concentration. Studies are currently in progress to delineate the effect of anthracyclines on mast cells.

In the present studies ADR and rubidazone caused rapid increases in plasma histamine levels and acute hypotension. This phenomenon was detected at ADR doses as low as 0.375 mg/kg. To the clinician, the differences in observed hypotensive potencies of ADR, rubidazone, and daunorubicin may not be meaningful because the latter two agents have been used at a dose two to three times higher than that of ADR. Thus, the therapeutic doses of these three compounds are similar with respect to their ability to produce hypotension. A good correlation has been found between the animal hypotensive responses and human clinical experience with regard to anthracycline-induced "allergic" phenomena. In one study "allergic" reactions were observed in 13% of 60 children receiving ADR (5). At least 15 other instances of "allergic-type" reactions following ADR administration have been reported to the Food and Drug Administration. Some of these reactions were systemic, leading in one instance to death. Similarly, fever, chills, hives, and shock have been reported to occur after rubidazone administration (16,17). The results of experimental studies further indicate that in clinical practice it should be possible to ameliorate the systemic effects of anthracycline-induced histamine release by extending the infusion period or, as suggested in a previous study (6), by pretreating the patient with antihistamine compounds.

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Phase I Toxicity Study of Methanol-Extracted Residue of Bacillus Calmette-Guérin by the Iv Route [1,2]

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SUMMARY

Sixty-four adult patients with a variety of disseminated malignant diseases received methanol-extracted residue (MER) of bacillus Calmette-Guérin (BCG) by the iv route in doses that ranged from 0.1 to 1.25 mg/m^2. Forty patients received iv MER alone weekly and 24 patients received MER combined with chemotherapy weekly. The number of doses given to each patient ranged from one to 18, with a mean number of doses of 2.3. Fever, invariably occurring at doses of > 0.5 mg/m^2, began around 3–6 hours with defervescence usually within 24 hours but lasting as long as 5 days. Chills, malaise, nausea and/or vomiting, and headaches were frequent side effects independent of dose. The large majority of patients had a good tolerance necessitating only symptomatic therapy. No difference in toxicity was found between the two groups analyzed. Eleven patients (17.2%) had more serious toxic effects; eight patients (13.7%) developed bilateral pulmonary infiltrates after one to 18 doses suggestive of granulomatous disease. This was not dose-related but correlated with history of prior BCG treatment or presence of a positive PPD skin test. The abnormality resolved spontaneously upon discontinuation of MER, usually within 4–6 weeks. There was one case of acute urticaria reaction controlled with antihistaminics. There was also one case each of acute erythrodermic rash and bullous dermatosis with acute vasculitis in the skin controlled with oral glucocorticoids. There were no major life-threatening toxic reactions and no long-term morbidity induced by iv MER. No definite tumor responses were documented. It appears that iv MER is a safe modality of treatment which should be investigated in phase II trials. The dose should be reduced in patients with a history of prior BCG or a positive PPD skin test. A dose of 0.1 mg is recommended for these patients and a dose of 0.5 mg/m^2 is recommended for the others.

[Cancer Treat Rep 63:1781–1786, 1979]

MER is the methanol-extracted residue of the Phipps strain of bacillus Calmette-Guérin (BCG). It is a nonviable vaccine that offers the advantage of accurate dose administration.

Animal studies with MER have demonstrated antitumor activity similar to that of BCG. It can protect against neoplastic isografts in mice (1), and can increase resistance to the leukemogenic action of radiation leukemia virus (2). When combined with chemotherapy or radiotherapy, MER caused inhibition of tumor and prolongation of survival superior to the other modalities of treatment alone (3). Furthermore, pretreatment of guinea

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pigs with MER gave protection against challenge with a transplantable hepatocarcinoma even though immunization and tumor challenge sites were different (4).

A number of phase I and II clinical trials of MER administered intradermally or sc in cancer patients have been reported. Chemotherapy and MER given intradermally to patients with acute myelogenous leukemia (AML) showed prolonged remission compared to patients receiving chemotherapy alone (5). An increased KLH antibody response and lymphocyte blastogenesis response to PHA compared to a control group was also noted (5). Therapeutic benefit was also demonstrated in AML by other investigators (6). Patients with advanced gastrointestinal adenocarcinoma receiving MER (intradermally) showed stimulation of cell-mediated immunity, particularly with the weekly schedule of administration (7,8).

Richman et al (9,10), using sc MER in patients with advanced solid tumors receiving a variety of chemotherapeutic agents, demonstrated an increase in skin test response to recall antigens and PPD and an increase in in vitro lymphocyte blastogenesis response. The addition of sc MER to combination chemotherapy resulted in prolongation of duration of remission and survival in patients with breast cancer (11,12).

On the other hand, in experimental tumor systems, iv immunotherapy has been shown to be superior to the intradermal or sc route. This was particularly evident in relation to visceral tumor involvement (especially lung) (13,14). Thus, iv immunotherapy is becoming increasingly important in clinical oncology. Israël et al (15) reported antitumor responses in patients with advanced solid tumors receiving daily iv *Corynebacterium parvum*. Preliminary results carried out at our institution have demonstrated increased response and survival in patients with malignant melanoma with *C. parvum* as compared to previous results with BCG scarification.

The above results prompted interest in the iv administration of MER. After the demonstration that iv MER was well-tolerated upon administration to the dog (16), we initiated a phase I study in cancer patients treated at our institution. The results of our study are the subject of this report.

MATERIALS AND METHODS

Patients

Sixty-four patients with histologically proven disseminated malignant disease were included in the study. Depending on whether MER was given alone or in combination with single-agent chemotherapy, the patients were subdivided into the two following subgroups: (*a*) 35 patients with advanced malignant disease (previously treated with a variety of chemotherapeutic regimens of higher priority) and five patients with a diagnosis of preleukemia (in whom chemotherapy was not indicated) received iv MER alone, and (*b*) 20 patients with advanced colon carcinoma who received vindesine as part of a randomized phase II study also received iv MER. Four more patients (two with melanoma and two with breast carcinoma) were given other chemotherapeutic agents together with iv MER (maytansine was given to three patients and combination chemotherapy was given to one).

MER Administration and Patient Monitoring

Both subgroups of patients were considered as one single group with regard to MER dose and escalation. MER was suspended in 150 ml of normal saline and was infused over 1 hour iv at weekly intervals. Patients receiving initial treatment and patients receiving new dose escalations were admitted to the hospital for treatment. However, with the lack of severe side effects, most patients were later treated on an outpatient basis. Vital signs were recorded at one-half-hour intervals during the first 6 hours and then every hour for the subsequent 6 hours. The patients' pulse and temperature and presence of chills were also recorded every 6 hours for an additional 48-hour period. Any evidence of fever, chills, changes in blood pressure, heart rate or respiratory symptoms, and any other subjective or objective symptoms or signs were recorded on special flow sheets. Fever was defined as a temperature of $> 37°C$ for > 1 hour. Acetaminophen was given for a temperature of $> 38°C$. The usual emergency drugs and instruments were always available at the bedside of the patient. The starting dose of MER was 0.01 mg/m^2 with escalation after two courses given at the same dose level. The schedule of dose escalation was as follows: 0.05, 0.1, 0.2, 0.4, 0.8, 1.0, and 1.25 mg/m^2 (highest dose given in the study). The number of courses ranged from one to 18, with a mean number of doses of 2.3 at the same level.

Laboratory and Immunologic Investigations

All patients had pretreatment chest roentgenogram, pulmonary function tests, cbc with differential and platelet count, coagulation profile, blood chemistry, serum electrolytes, and ECG. These tests were repeated at regular intervals, after each course, and whenever additionally indicated.

All patients had delayed hypersensitivity skin test reactions to a battery of recall antigens consisting of dermatophytin, *Candida*, Varidase, mumps, KLH, and PPD prior to initiation of therapy. Serial immunologic evaluation in selected patients is the subject of a separate report.[5]

[5] Hersh EM, Murphy SG, Quesada JR, et al. The effect of immunotherapy with *C. parvum* and MER administered intravenously on host defense function in cancer patients. Submitted for publication.

TABLE 1.—Characteristics of patients receiving iv MER

Characteristic	Treatment: MER alone	Treatment: MER + chemotherapy
No. of evaluable patients	40	24
Male/female ratio	28/12	13/11
Median age in yrs (range)		
Males	48 (23–73)	55 (30–68)
Females	43 (30–71)	59 (37–80)
No. of evaluable courses	96	105
No. of patients with—		
Prior BCG	8	4
Pretreatment PPD negative	30	17
Pretreatment PPD positive (induration [mm] at 48 hrs)		
10–15	2	5
> 15	8	2

TABLE 2.—Diagnoses in patients receiving iv MER alone

Diagnosis	No. of patients
Malignant melanoma	15
Lung carcinoma	6
Colon carcinoma	4
Smoldering leukemia	5
Sarcomas	3
Renal cell carcinoma	2
Carcinoid tumor	1
Thymoma	1
Esophageal carcinoma	1
Non-Hodgkin's lymphoma	1
Malignant fibrous histiocytoma	1
Total	40

RESULTS

The two groups of patients were analyzed separately. Table 1 shows characteristics of each group and table 2 shows the diagnoses made in patients receiving iv MER alone. Table 3 includes the number of patients receiving each dose, the number of evaluable courses, and the number of courses without fever for both groups of patients. At the lower doses, 32% of the courses were not associated with fever, whereas at doses of > 0.5 mg/m^2, fever occurred with almost all courses of therapy.

For both groups, the median height of fever was quite similar within all dose levels (38°C–39°C) as was the temperature range (37°C–40.5°C). The majority of patients recorded low-grade fever (37°C–37.9°C) at some point in time and hence temperatures of > 37°C were included in the analysis. In addition, onset of fever did not differ significantly between the two groups, occurring usually 3–6 hours after the infusion. The median duration of fever ranged from 18 to 48 hours within all dose levels, without any consistent dose-related pattern. Only those patients receiving iv MER alone at doses of < 0.2 mg/m^2 showed a difference. The median durations of fever were 65, 60, and 69 hours for 0.01, 0.05, and 0.1 mg/m^2 dose levels respectively. These groups clustered more patients with positive PPD (5) and prior BCG therapy (5) which could explain the difference. It is noteworthy that patients at the same dose levels receiving chemotherapy had a median duration of fever of 30 hours.

The incidence of other symptoms and signs are shown in tables 4 and 5 according to three different dose ranges. In the majority of patients these side effects were not severe and necessitated only symptomatic therapy. Dyspnea was a transient subjective symptom in most of the patients. Two patients complained of arthralgias and myalgias. Patients with positive PPD or previous exposure to BCG tended to have more severe reactions, and

TABLE 3.—Evaluable courses/dose level

Dose level (mg/m^2)	No. of patients receiving— MER alone	No. of patients receiving— MER + chemotherapy	No. of evaluable courses: MER alone	No. of evaluable courses: MER + chemotherapy	No. of courses without fever: MER alone	No. of courses without fever: MER + chemotherapy
0.01	6	5	10	17	5	6
0.05	4	8	8	32	4	14
0.1	5	6	7	15	3	4
0.2	4	7	11	23	3	4
0.3	1	0	4	—	0	—
0.4	3	2	11	4	3	1
0.5	2	0	5	—	0	—
0.8	5	4	10	12	0	1
1.0	6	2	14	2	0	0
1.25	9	0	16	—	1	—

TABLE 4.—Symptoms and signs associated with iv MER alone

Dose level (mg/m^2)	No. of patients	No. of evaluable courses	No. of patients (%) with—			
			Chills	Nausea and/ or vomiting	Malaise	Respiratory symptoms
< 0.1	15	24	8 (33)	10 (42)	10 (42)	10 (42)
0.2–0.5	10	31	19 (61)	6 (19)	17 (55)	4 (13)
0.8–1.25	20	36	23 (64)	12 (33)	23 (64)	23 (64)

TABLE 5.—Symptoms and signs associated with iv MER + chemotherapy

Dose level (mg/m^2)	No. of patients	No. of evaluable courses	No. of patients (%) with—			
			Chills	Nausea and/ or vomiting	Malaise	Respiratory symptoms
< 0.1	19	64	17 (26)	16 (25)	21 (33)	9 (14)
0.2–0.4	9	27	4 (15)	3 (11)	9 (64)	2 (7)
0.8–1.0	6	14	8 (57)	7 (50)	11 (78)	5 (35)

dose reduction was needed more frequently, but no statistically significant difference was found. The incidence of side effects increased at doses of > 0.8 mg/m^2 in both groups. Chills and malaise also tended to be more intense. In particular, dyspnea and cough were more common at these dose levels. There was no evidence of cumulative toxicity with up to 18 consecutive doses of iv MER. In most of the patients MER was discontinued upon disease progression.

Toxicity Necessitating Reduction or Discontinuation of MER

Eleven of 64 patients (19%) developed significant toxic effects following iv MER which necessitated discontinuation of the drug in nine patients and dose reduction with better tolerance in the remaining two patients. Details regarding these patients and the type of dose-limiting toxicity encountered are shown in table 6.

The most serious complication was bilateral pulmonary infiltrates suggestive of granulomatous disease. In eight patients (12.5%) the appearance of the chest roentgenogram and the time relationship to MER administration was very suggestive of a causal relationship. Five patients complained of dyspnea and two had dry cough. Two patients had documented hypoxemia. However, the mild nature of the symptoms never warranted an open lung biopsy when each patient was judged individually. Thus, no histologic diagnosis was made. Changes mimicking miliary tuberculosis usually occurred after three or more courses of MER, but were not dose-related. Progressive resolution of the infiltrates was noticed in all of the patients upon discontinuation of MER, without any further treatment.

Four of ten patients with prior exposure to BCG (40%) and six of 19 patients with positive PPD skin tests (31%) developed this complication, compared to four of 49 patients without BCG ($P < 0.01$) and two of 40 patients with negative tuberculin reaction ($P < 0.01$). A detailed description of these patients can be found elsewhere (17).

Three different patterns of acute dermatologic reaction were seen in three patients. One patient presented with acute urticaria which was well-controlled with antihistaminics, another patient had an acute erythrodermic rash which regressed when oral glucocorticoids were given, and the last patient had an acute bullous dermatosis resembling erythema multiforme and involving oral mucosa. Biopsy showed acute vasculitis. Slow regression of the lesion also occurred with steroids. Two more patients developed reactivation of previous BCG scarification sites and of previous intradermal hypersensitivity skin tests respectively.

Response

No objective responses were noted in patients receiving iv MER alone. In one patient with pulmonary metastases who developed lung infiltrates, one of the pulmonary lesions showed slight regression whereas the other previous lesions remained stable for 3 months. Interestingly, this patient's skin lesions progressed while the pulmonary lesions remained stable. The same phenomenon was also noted in another patient.

DISCUSSION

In this phase I toxicity study, iv MER was well-tolerated overall. This has also been demonstrated by other

TABLE 6.—Dose-limiting toxicity associated with iv MER

Patient age/sex	Diagnosis	Prior BCG	PPD induration (mm) at 48 hrs	Dose (mg/m^2) of MER given × No. of courses	Toxic effects
43/F	Breast cancer	No	17 × 18	0.4 × 2 0.2 × 1	Severe malaise, dyspnea, bilateral pulmonary infiltrate on chest roentgenogram; spontaneous resolution
38/M	Malignant melanoma	Yes	18 × 18	0.05 × 1 0.025 × 4	Severe malaise, fever of > 40°C, skin reaction at prior BCG sites, bilateral pulmonary infiltrates and mild hypoxia; resolving
43/M	Malignant melanoma	Yes	48 × 35	0.2 × 1	Severe malaise, fever of > 39.5°C, bilateral pulmonary infiltrate on chest roentgenogram; MER discontinued: symptoms subsided in < 1 wk
48/F	Lung cancer	No	Negative	0.8 × 1 0.5 × 2 0.3 × 2 0.2 × 3	Severe malaise, flu-like symptoms, headache; MER initially reduced, then discontinued
32/F	Preleukemia	No	23 × 20	0.1 × 1 0.5 × 4	Miliary-type infiltrate on chest roentgenogram; spontaneous resolution
57/M	Colon carcinoma	No	Negative	0.1 × 2 0.2 × 16	Bilateral lung infiltrates; spontaneous resolution
50/F	Leiomyosarcoma	Yes	20 × 18	0.01 × 5	Bilateral multiple nodular densities; spontaneous resolution
41/M	Malignant melanoma	Yes	40 × 30	1.25 × 1	Acute erythematous rash, severe side effects, pulmonary infiltrates; slow resolution
53/M	Preleukemia	No	Negative	0.5 × 1	Cutaneous vasculitis, bullous dermatitis
46/M	Thymoma	No	Negative	1.0 × 3	Acute allergic urticarial reaction; drug dose reduced with better tolerance: symptoms subsided in < 3 days
56/M	Malignant melanoma	No	Negative	0.1 × 1 0.2 × 5	Severe malaise and weakness, fever of > 40°C; MER dose reduced with better tolerance: symptoms subsided in < 1 wk

investigators (18,19). In the present series, the most common reaction to MER was fever which was always present at the higher doses of the drug. Fever tended to start within 5 hours of administration and usually lasted for a period extending to an average of 48 hours. Chills, nausea and/or vomiting, headache, myalgias, and other symptoms tended to be moderate, necessitating only symptomatic treatment.

The most significant factor limiting dose and duration of therapy has been the development of bilateral pulmonary infiltrates suggesting miliary granulomatous changes. Unfortunately, this was not confirmed histologically. However, it is noteworthy that two well-documented cases reported by Lundy et al (20) and Voith et al (21) described the occurrence of pulmonary granulomas in patients receiving intradermal and intratumoral MER. In view of the prior description of such a complication with BCG therapy (22), the occurrence of pulmonary granulomas with iv MER therapy is not totally unexpected. Moreover, most of the patients developing this complication had a strongly positive PPD reaction and/or had received prior BCG therapy. In such patients special caution is recommended when iv MER is given, and regular followup and serial chest roentgenograms are indicated. So far, spontaneous resolution has occurred upon discontinuation of the agent in all of our patients, and no treatment seems to be needed. MER did not have any untoward effect on serial biochemical studies (particularly renal and hepatic functions) or blood cell counts. On the contrary, patients receiving vindesine and MER seemed to have a better tolerance to dose escalation of chemotherapy, and preliminary unpublished in vitro studies seem to indicate that MER causes an increase in the colony-stimulating factor. This could be relevant to patients receiving a combination of chemoimmunotherapy and supports the use of MER in patients with bone marrow aplasia or dysplasia.

In this study, we have attempted to determine the maximum-tolerated dose of iv MER. It appears that iv MER is a safe modality of treatment. A dose of 0.5 mg/m^2/week is well-tolerated by the majority of patients but should be reduced to 0.1 mg/m^2 in patients with prior

BCG or a positive PPD skin reaction, who tend to show more intense side effects and higher risk of pulmonary toxicity. However, these doses do not necessarily correlate with the optimum dose from an immunologic point of view. We are presently in the process of undertaking extensive immunologic evaluation of patients receiving iv MER in an attempt to determine the optimum dose and schedule of the drug prior to initiation of phase II clinical studies of the drug.

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Sorbent Removal of Adriamycin In Vitro and In Vivo [1,2,3]

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SUMMARY

Adriamycin adsorption to four sorbents, uncoated activated charcoal, acrylic hydrogel-coated activated charcoal, a polystyrene non-ionic resin, and a pyrolized resin, was determined in vitro in aqueous and plasma solutions. Uncoated charcoal and pyrolized resin were most efficient in removing Adriamycin from these solutions. Four hours after an iv bolus of Adriamycin (2.5 mg/kg) to beagle dogs, acrylic hydrogel-coated activated charcoal hemoperfusion was performed. Extraction of Adriamycin averaged 43% throughout the 3-hour hemoperfusion period and resulted in a 20-fold increase in total body elimination of Adriamycin, but plasma drug concentrations returned towards control values following hemoperfusion. Pharmacokinetic analysis of plasma Adriamycin profiles suggested that reduction of tissue concentrations of Adriamycin could be attained if hemoperfusion periods were extended. It is suggested that hemoperfusion may be useful in reducing blood levels of Adriamycin in patients with hepatic disease or accidental overdosage, but that reduction of tissue levels of Adriamycin would require extended or repeated hemoperfusion treatments.

[Cancer Treat Rep 63:1787–1793, 1979]

Adriamycin is an anthracycline anticancer agent with a broad spectrum of activity for human malignancies (1). However, Adriamycin produces acute hematologic and mucosal toxicity, as well as treatment-limiting damage to the myocardium at total cumulative doses in excess of 500 mg/m^2, or at lower doses in patients with prior mediastinal radiation (2,3). Adriamycin is metabolized in the liver and depends upon biliary excretion as its principal route of elimination (4,5). In patients with impaired liver function or biliary obstruction, cytotoxic blood levels are maintained for excessive periods with resultant severe, and potentially lethal, acute toxicity (6).

The application of sorbent hemoperfusion for drug intoxication, other than anticancer drug intoxication, has shown that hemoperfusion of blood through activated charcoal or resin preparations is an efficient and simple technique, particularly useful in removing drugs which are inefficiently removed by standard hemodialysis (7). In previous studies we have shown that hemoperfusion can increase the naturally occurring elimination rate of drugs from experimentally intoxicated animals (7,8). Prevention of the acute adverse effects of Adriamycin might be possible if the rate of elimination of the drug could be enhanced by an auxiliary method. This report deals with the effects of sorbent removal of Adriamycin from aqueous and biologic solutions in vitro and the results of

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TABLE 1.—Characteristics of sorbents

Sorbent	Average pore size (Å)	Manufacturer	Supplier
Coconut shell, activated charcoal (mesh size, 5–10)	40	Sutcliffe-Speakman, England	Warner Lambert Research Institute, US
2% acrylic hydrogel-coated coconut shell (mesh size, 5–10)	40	Sutcliffe-Speakman, England	Warner Lambert Research Institute, US
Cross-linked polystyrene non-ionic resin (XAD-4)	50	Rohm and Haas, US	Extracorporeal Medical Specialties, Inc, US
Pyrolized resin (XE-344)	49	Rohm and Haas, US	Extracorporeal Medical Specialties, Inc, US

sorbent hemoperfusion on removal of Adriamycin administered to beagle dogs.

MATERIALS AND METHODS

In Vitro Experiments

Adriamycin was dissolved in water (1 mg/ml); subsequent dilutions of stock solution were made in phosphate buffered saline (PBS), pH 7.4, to give concentrations of 10^{-6} M, 10^{-5} M, and 10^{-4} M Adriamycin. The sorbents used in the experiments are shown in table 1. Sorbent ranging from 1 to 7.5 g was weighed in an Erlenmeyer flask to which 25 ml of Adriamycin/PBS solution was added. The flasks were then transferred to a metabolic bath maintained at 37°C and shaken in the dark. In one series of experiments, supernatant was sampled for up to 1 hour and in another series samples were taken after 4 hours of incubation. One ml of the solution was transferred to glass tubes, diluted with PBS solution, and read in a Perkin-Elmer spectrofluorometer at 480 nm excitation and 590 nm emission. Fresh Adriamycin standard solutions were prepared in PBS and were used to prepare standard curves of total fluorescence against concentration of Adriamycin and to calculate the concentration of Adriamycin after adsorption to sorbents.

Adriamycin was added to human plasma to yield a concentration of 10^{-4} M. Twenty-five ml of plasma was transferred as in the above experiments to flasks containing graded amounts of charcoal and shaken at 37°C. After 4 hours, 1-ml samples were taken and pipetted into glass tubes, mixed with 0.3 N HCl and 50% ethyl alcohol, and extracted according to a minor modification of the procedure of Bachur et al (9). Adriamycin standards in plasma were mixed with 3 volumes of 0.3 N HCl and 50% ethanol and shaken vigorously. After the gel mixture was centrifuged at 20,000 × *g* for 20 minutes, total drug fluorescence of the supernatant was determined in a Perkin-Elmer spectrofluorometer. Recovery was > 95%. Standard curves of relative fluorescence versus concentration of Adriamycin were constructed and the amount of Adriamycin in plasma was determined after adsorption to charcoal.

To determine the stability of Adriamycin under the conditions of sorbent extraction, the drug was placed in PBS and in plasma at a concentration of 326 μg/ml (6×10^{-7} M) and incubated at temperatures of 4°C, 25°C, and 37°C. The concentration of Adriamycin was then determined as described above.

In Vivo Experiments

Female beagle dogs weighing 11–13 kg were used in these experiments. Four dogs were used as Adriamycin controls and were anesthetized and maintained under anesthesia with sodium pentobarbital. A jugular venous sampling line was placed and Adriamycin (2.5 mg/kg) in saline was administered by rapid iv injection using a peripheral limb vein. Timed blood samples were taken from the jugular line up to 72 hours after administration. The four dogs were then allowed to recover from anesthesia after drug administration and were cared for in our animal resources center.

Two of the control dogs, 6 weeks after the initial experiments and after complete recovery, and two previously untreated dogs were used in the hemoperfusion experiments. In addition to the placement of the jugular venous sampling line, while the animals were anesthetized femoral arterial and venous catheters were placed and used as respective inlet and outlet lines to the hemoperfusion column (fig 1). Blood was anticoagulated with intermittent administration of heparin to maintain a whole blood clotting time of 30–40 minutes.

A column containing 300 g of sterilized acrylic hydrogel-coated charcoal identical to that used in the in vitro experiments (Warner Chilcott, Morris Plains, NJ) was used in all the hemoperfusion experiments. Because of the rapid fall in plasma Adriamycin observed in the control experiments, hemoperfusion was instituted in all animals 4 hours following bolus administration of Adriamycin

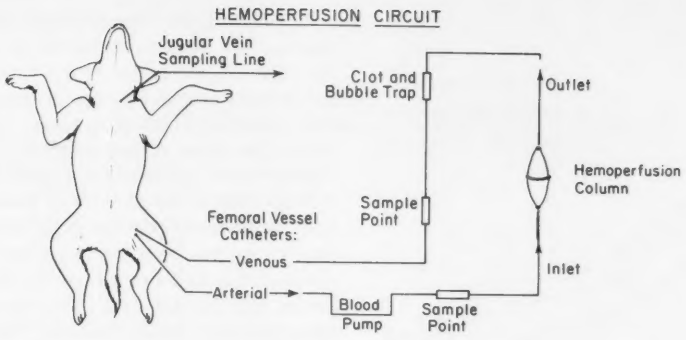

FIGURE 1.—In vivo hemoperfusion circuit.

(2.5 mg/kg). Blood flow was maintained throughout the 3-hour hemoperfusion period at 100 ml/minute and timed blood samples were taken from inlet and outlet blood lines attached to the hemoperfusion column (fig 1). Plasma Adriamycin "equivalent" was assayed by a modification of the spectrofluorometric method of Bachur et al (9). Plasma clearance of Adriamycin during hemoperfusion was calculated as described previously by us (8,10) using the formula

$$\text{clearance (ml/minute)} = \text{blood flow rate (ml/minute)} \times \frac{\text{inlet concentration} - \text{outlet concentration}}{\text{inlet concentration}}$$

The effect of hemoperfusion on whole body clearance of Adriamycin was assessed by subjecting the plasma concentration data to pharmacokinetic analysis using the NONLIN computer program of Metzler.[7] Visual inspection of the data showed that a model with more than one compartment was required to adequately describe the pattern of fall in plasma concentration. A comparison of goodness of fit of control data to a two- or three-compartment model showed the latter to give the better fit ($r^2 = 1.000$). Accordingly, the three-compartment model in which drug is considered to be distributed between plasma and two tissue compartments was used in subsequent analyses. Irreversible losses from plasma were described as clearance, related by a volume term to the size of the plasma compartment. The distribution between plasma and the shallow (or more rapidly equilibrating) and deep (or more slowly equilibrating) compartments was described by first-order rate constants.

From the derived parameters the effects of hemoperfusion on elimination and distribution of Adriamycin were computed. The combined effects are described as exposure of the various compartments to the drug, ie, as the fraction of the body burden in each compartment and as the product of the mass of drug and duration of its residence in each compartment. This approach has been used by Riggs and colleagues (11). In the hemoperfusion experiments, total body clearance during hemoperfusion was taken as the sum of the natural, pre-perfusion clearance and the measured clearance by the column.

RESULTS

At 4°C and 25°C, no decomposition of Adriamycin was observed after 48 hours of incubation. However, after 4 hours of incubation at 37°C in PBS, 88% of Adriamycin was recovered, decreasing to 40% after 24 hours and 24% after 48 hours. However, comparable experiments performed with the drug in plasma demonstrated that only 4% of Adriamycin was decomposed after 24 hours of incubation. In view of the loss of Adriamycin in PBS, true Freundlich or Langmuir isotherms could not be constructed.

Adriamycin adsorption from PBS with 1 g of each of the sorbents is shown in figure 2. Percentage removal was calculated by comparing the amount remaining in the sorbent supernatant with that in a control flask not containing sorbent. This shows that Adriamycin is efficiently and rapidly removed by all sorbents and that uncoated charcoal and XE-344 pyrolized resin were the most efficient. At concentrations of 10^{-5} M and 10^{-6} M Adriamycin adsorption was complete. Adriamycin adsorption from plasma at 37°C was performed at a concentration of 10^{-4} M. Figure 3 shows Adriamycin uptake from plasma with increasing weights of each of the four sorbents. Again, uncoated charcoal and XE-344 are the most efficient sorbents, but their efficiency is somewhat reduced by the presence of other components in the plasma. This represents about 50% Adriamycin adsorption from plasma by 1 g of the two sorbents, whereas comparable experiments in PBS demonstrated complete adsorption.

[7]Metzler CM. NONLIN—a computer program for parameter estimation in non-linear situations. Technical report No. 7292/69/7292/005. Kalamazoo, MI, The Upjohn Co, Nov 25, 1969.

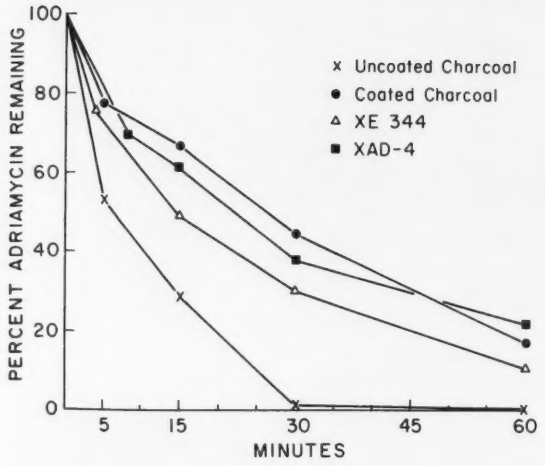

FIGURE 2.—Adsorption of Adriamycin (10^{-4} M) from PBS (pH 7.4) with 1 g of sorbents at 37°C.

Results of the in vivo experiments are shown graphically in figure 4. This shows that not only are plasma concentrations of Adriamycin substantially lowered by hemoperfusion, but coated charcoal continues to be highly adsorptive for Adriamycin throughout the experimental period, as shown by the inlet-outlet concentration differences. The mean plasma clearance of Adriamycin during hemoperfusion was 43.0 ± 5.6 ml/minute. Following hemoperfusion (24 hours after iv bolus administration) plasma Adriamycin concentrations in two dogs (samples were not obtained in the other two dogs) demonstrated a "rebound" toward the pre-perfusion values, although the concentrations were substantially lower than the earlier levels.

Although three compartments were required to best describe the plasma concentration data, the plasma and the shallow tissue compartment were in rapid equilibrium and could be considered together, with tissue distribution then referring to the deep tissue compartment. Table 2 shows the effect of hemoperfusion on distribution and elimination of Adriamycin. Computer-simulated results indicate that in the absence of hemoperfusion, 98.1% of body Adriamycin is in the deep tissue compartment and only 0.4 mg has been eliminated in 6 hours with 0.52 mg eliminated in 12 hours, the decline in plasma levels being almost entirely due to distribution to the tissues. With hemoperfusion, mean clearance was 3.0 liters/hour at 4 hours, which represents a 20-fold increase compared to controls. At 6 hours after hemoperfusion, 6.2 mg of Adriamycin was eliminated and hence it can be predicted that 17.4 mg of the drug would have been removed by 12 hours if hemoperfusion had been continued. The computer-assisted data show that redistribution has occurred with 99.4% of the body levels now in tissues. Thus, there has been a marked reduction in the plasma concentrations, partly the result of increased elimination and partly the result of redistribution.

The "exposure" of the plasma to Adriamycin in the 4–6-hour period after injection is 0.468 μg·hour in the absence of hemoperfusion and 0.157 μg·hour in the presence of hemoperfusion at a mean clearance of 3.0 liters/hour. That is, the overall exposure of the plasma compartment has been reduced to 33.5% of control values. Considering the plasma and shallow tissue compartment as one, then the overall exposure during hemoperfusion is reduced to 39.2% of that in controls. Less dramatic changes are seen in the deep tissue compartment, where the exposure is only reduced to 90.5% of the control value. An accelerated decline in tissue levels could be seen on the simulated computer program (fig 5). Predictions made on the basis

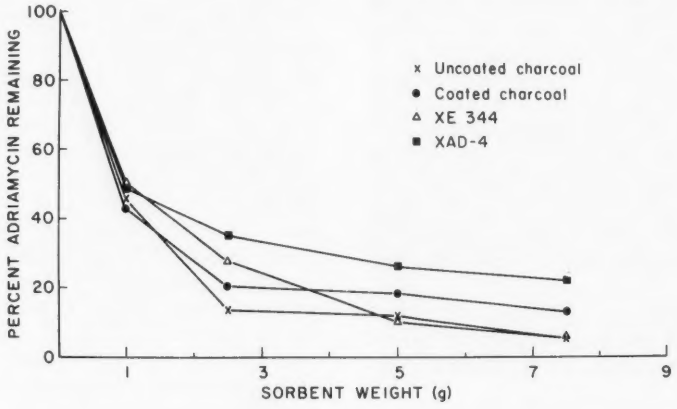

FIGURE 3.—Adsorption of Adriamycin (10^{-4} M) from plasma with varying sorbent weights at 37°C (4 hrs).

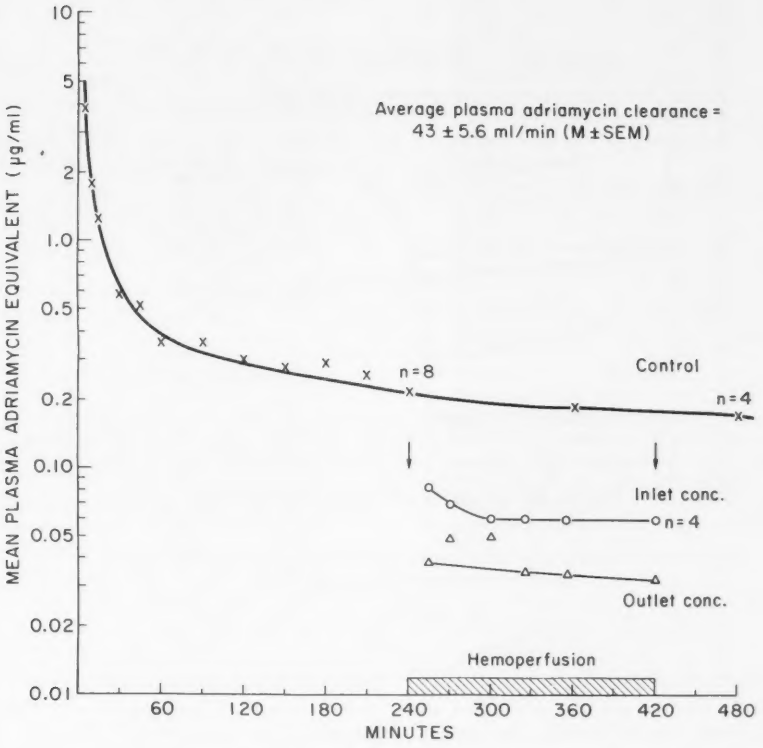

FIGURE 4.—Mean plasma Adriamycin equivalent concentration in control and hemoperfused beagle dogs.

of these data show that the hemoperfusion time should be lengthened to 6 hours and/or the blood flow rates increased from 200 ml/minute to 300 ml/minute in order to reduce tissue concentrations.

In these experiments only one death in the control series and no deaths in the test animals occurred. Platelet counts were reduced by 25%–30% in the hemoperfused dogs but returned to pre-perfusion levels within 48 hours.

DISCUSSION

The presence of hepatic or renal dysfunction in patients with cancer effectively limits or prohibits therapy with specific anticancer drugs that are eliminated by urinary or biliary excretion. While reduction of the dose of a chemotherapeutic agent may reduce acute drug toxicity, accidental overdosage or drug-induced hepatic or renal dysfunction may result in cumulation of drug to toxic levels. A technique which reduces concentration × time of exposure to tissues of anticancer drugs could have an impact on the treatment of patients with organ dysfunction. Adriamycin is a broad-spectrum chemotherapeutic agent which is eliminated largely by biliary excretion. Significant hepatic dysfunction seriously limits patient tolerance to the administration of this agent. However, it is possible that therapy could be instituted safely if an auxiliary mode of Adriamycin removal were available.

TABLE 2.—Effect of hemoperfusion on in vivo distribution and clearance of Adriamycin equivalents

	Column clearance (liters/hr)	Total clearance (liters/hr)	F1*	F2	F3
Control	—	0.156	0.8	1.1	98.1
Hemoperfusion	3.0	3.09	0.3	0.4	99.4

*F1, F2, and F3 are respective fractions (%) of total body Adriamycin equivalents in the plasma compartment, the rapidly equilibrating (shallow) compartment, and the deep tissue compartment.

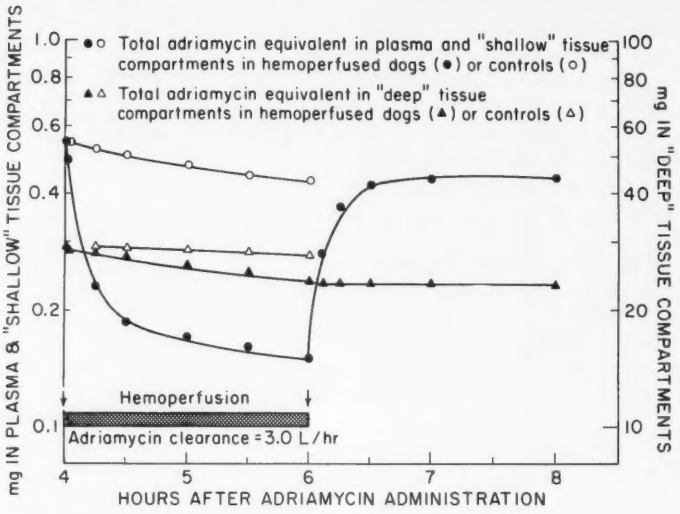
mg IN PLASMA & "SHALLOW" TISSUE COMPARTMENTS
mg IN "DEEP" TISSUE COMPARTMENTS
●○ Total adriamycin equivalent in plasma and "shallow" tissue compartments in hemoperfused dogs (●) or controls (○)
▲△ Total adriamycin equivalent in "deep" tissue compartments in hemoperfused dogs (▲) or controls (△)
Hemoperfusion
Adriamycin clearance = 3.0 L/hr
HOURS AFTER ADRIAMYCIN ADMINISTRATION

FIGURE 5.—Computer-simulated values of Adriamycin equivalents in tissues of control or hemoperfused beagle dogs.

In vitro, all of the sorbents were efficient in removing Adriamycin. Uncoated charcoal and XE-344, even at weights of 1 g, could remove up to 50 times the normal plasma therapeutic level of Adriamycin (5). The presence of a 2% acrylic hydrogel coating on charcoal does slow the rate of adsorption of Adriamycin.

Adsorption of Adriamycin from plasma solutions is reduced by the presence of plasma components. This may represent saturation of sites by preferential adsorption, a well-recognized phenomenon. Even though differences in adsorption existed between the sorbents, we chose for our in vitro experiments a cartridge containing acrylic hydrogel-coated charcoal. The reasons for this choice are as follows: (*a*) the column is widely available in the United States; (*b*) the total capacity for drug adsorption is not decreased by the presence of an acrylic hydrogel coating, although the rate of drug adsorption is somewhat decreased (12) as we had demonstrated in our in vitro experiments; and (*c*) the compatibility of the surfaces through which blood is passed gives fairly predictable and acceptable platelet losses in man (13).

In general the anthracyclines possess a long plasma half-life which might offer the serious disadvantage of toxicity to myeloproliferative and cardiac cells. Hemoperfusion over charcoal in vivo caused an increase in body clearance of Adriamycin equivalent of the order of 20-fold. The increased clearance together with redistribution during hemoperfusion resulted in a marked lowering of Adriamycin levels in plasma and rapidly equilibrating tissues, the mean exposure in a 2-hour period of hemoperfusion being 39.2% of that in controls. The exposure of deep tissues to Adriamycin was reduced to 90.5% of controls in the same period.

As seen from the computer predictions, and observed in vivo, one result of slow equilibration of Adriamycin between the "shallow" and "deep" tissue compartments is that Adriamycin levels in blood can be expected to rise after hemoperfusion is discontinued. Such increases in Adriamycin levels may not be adverse since they take place at the expense of concentrations of Adriamycin in the more slowly equilibrating "deep" tissue compartment. Kinetic analysis of our results has shown that reduction of "deep" tissue levels of Adriamycin is attainable. However, this would require that hemoperfusion times be lengthened or that hemoperfusion be repeated at intervals. These results are similar to previously reported observations of digoxin pharmacokinetics in dogs treated with hemoperfusion (14).

These preliminary experiments show that hemoperfusion is an effective method for the removal of plasma Adriamycin and could result in lowered tissue levels. It is estimated that lowered tissue levels could be achieved if the hemoperfusion period was prolonged or if blood flow rates were increased. We did not attempt to address the question of whether reduction of tissue or plasma Adriamycin levels reduced Adriamycin toxicity in this series of experiments. Such observations are now warranted before hemoperfusion for Adriamycin removal can be recommended in situations such as accidental overdosage or impaired hepatic function in man.

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Utilization of Metabolic Activation for In Vitro Screening of Potential Antineoplastic Agents [1,2]

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Several chemotherapeutic agents used clinically or under investigation for potential use in cancer treatment must be metabolically altered from their initial chemical form to a biologically active state. Clinically employed chemotherapeutic agents that require metabolic activation include cyclophosphamide (1–4) and DTIC (5–7). Several potential anticancer compounds that appear to require metabolic activation are hexamethylmelamine (8–10), pentamethylmelamine (11), and the antimetabolite, 2-amino-1,3,4-thiadiazole (12).

As part of the National Cancer Institute's (NCI) program to develop new antineoplastic agents, crude plant extracts are screened for potentially active materials in a transplantable animal tumor model (P388 mouse lymphocytic leukemia) and in a human tumor cell culture system (KB human epidermoid carcinoma). Materials found active in the in vitro KB cell culture system are subsequently fractionated, and the KB cells are used to follow the isolation of the active fractions. Those crude plant extracts found active in vivo are tested for activity against KB cells in vitro and, if inactive against the latter, the extracts are also tested against P388 and L1210 cells in vitro to determine if one of these cell lines can be used to follow activity during fractionation. Often, however, materials which are active in the in vivo system are found to be inactive in all three in vitro systems. The purification of the active component(s) of these materials is then quite difficult and expensive, since the in vivo system must be used for the bioassay. Several authors (13–15) have suggested that a major limitation of in vitro systems is their inability to detect those materials whose cytotoxic effects are due to metabolic products formed by enzymes not present in the cultured cells, but present in the animal model (eg, hepatic enzymes).

We have extended the work of Dolfini et al (13) in associating an in vitro metabolic activation system with cultured KB cells in an attempt to increase the detection capabilities of the in vitro test system. In our KB test system with metabolic activation, we have examined five clinically used antineoplastic agents which were reported to require metabolic activation, as well as ten materials which were active in the NCI in vivo test system but inactive in vitro. The usefulness of metabolic activation in cell culture screening for potential antineoplastic agents, for the isolation of active components of in vivo active crude materials, and for the study of mechanisms of action of antitumor agents is discussed in light of our results.

MATERIALS AND METHODS

S-9 Fraction

The S-9 fraction was prepared from Aroclor 1254-induced male $CD2F_1$ mouse liver by the method of Ames et al (16) or was purchased commercially from Litton Bionetics, Inc (Kensington, MD) (Catalog No. 8360), where the S-9 fraction is prepared from Aroclor 1254-induced male Sprague-Dawley rat liver by the method of Ames et al (16).

Maintenance of KBE Human Epidermoid Carcinoma Cells in Culture

The KBE cell line was first isolated in 1954 from a male Caucasian with an ulcerated friable tumor of the mouth and tongue (17). The line of KBE cell stock maintained in our laboratory was originally obtained from Arthur D. Little, Inc (Cambridge, MA).

The KB cells are grown surface-attached in Pyrex Blake bottles. They are maintained in Eagle's basal medium (diploid) with Earle's salts (BME) supplemented with 10% heat-inactivated fetal calf serum (Reheis Chemical Co, Kankakee, IL), 50 units/ml of penicillin, and 50 μg/ml of streptomycin (Grand Island Biological Co, NY). The BME growth medium is obtained in powdered form and reconstituted in 10-liter quantities. A Millipore Twin-90 filter is used to sterile filter each lot; all of the 500-ml bottles prepared are incubated for 24 hours in a warm

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room (37°C) as a sterility check prior to the addition of antibiotics.

The passage of the cells into fresh bottles is performed biweekly by first removing the growth medium and washing the attached cells two times with Pucks' Saline A. The cells are then trypsinized with a 10-ml volume of a 0.05% trypsin-0.02% EDTA solution for 1½ minutes. All but 1 ml of the trypsin-EDTA solution is then removed, and the bottle is allowed to stand at room temperature for an additional 1–2 minutes until the cell sheet begins to detach. After several sharp raps on the side of the bottle to dislodge the remaining cells, 9 ml of fresh warm BME with serum is added to inactivate the trypsin. The 10-ml cell suspension is then pipetted up and down ten times to declump the cells. A 0.2-ml aliquot is removed for determination of the cell concentration by a Coulter count (Model ZBI Coulter Counter) and aliquots containing 3×10^6 cells are pipetted into two fresh bottles containing 75 ml of complete BME. The bottles are gassed for 1 minute with 5% CO_2/95% air, and are maintained stationary on the shelf in the walk-in warm room at 37°C. The passaging is performed regularly on Monday and Thursday. Blake bottles for experimental use are seeded on Monday with 3.0×10^6 cells and are re-fed on Thursday for use on Friday.

Modification of the Standard KBE Assay

The assays are performed in 16 × 150-mm sterile, silicone-rubber-stoppered, disposable culture tubes in stainless steel spring-loaded racks. A cell suspension is prepared by trypsinizing cells in a Blake bottle in a proliferative (nonconfluent) stage of growth. Fresh medium is added to the cell culture 24 hours before use. The trypsinized cells are counted and the appropriate number is resuspended in warm BME containing 10% fetal calf serum, 50 units/ml of penicillin, and 50 μg/ml of streptomycin so that the cell concentration is 17,000 cells/ml. Four-milliliter aliquots of the stirred cell suspension are then distributed into each tube with a Cornwall automatic syringe and they are allowed to incubate at a 10° angle at 37°C for 48 hours. After 48 hours, the medium is aspirated and treatment is initiated by the addition of the following solutions which are prepared in BME supplemented with 2% fetal calf serum just prior to use and added to the tubes in the following order listed.

(*a*) *Medium (2% fetal calf serum)*—medium is added as required so that the total volume of each tube will be 4 ml after the addition of the appropriate amount of each of the following solutions.

(*b*) *Cofactors and S-9 fraction*—2 ml of medium containing 4 mg of NADP, 30 mg of glucose-6-phosphate, and 0.2 ml of an S-9 preparation whose concentration is approximately 40 mg of protein/ml.

(*c*) *Test agent*—0–2 ml of medium containing the appropriate amount of test agent.

After the addition of the treatment medium, the tubes are stoppered and gassed for 5 seconds with 95% O_2/5% CO_2 flowing at 12 liters/minute. They are then incubated at 37°C for 3 hours while rocking front to back on a Belco rocking platform at 5 cycles/minute. Following incubation, the treatment medium is aspirated and the cell sheets are washed twice with 4 ml of BME containing 2% fetal calf serum. BME (4 ml) containing 10% fetal calf serum is then added to each of the tubes and they are incubated for an additional 21 hours. The total incubation time is 72 hours. The tubes are then removed from the warm room and the medium is decanted over the side opposite the cell sheet. The racks are inverted and allowed to drain on absorbent paper momentarily before the cells are washed twice with 5 ml of warm Earle's balanced salt solution minus bicarbonate. They are then allowed to drain an additional 15 minutes before 10 ml of Lowry's C reagent (18) is added to each tube to dissolve the cells. The racks are then stoppered with clean silicone stoppers, secured between two boards joined at the corner with screws, and placed on a reciprocating shaker at 100 strokes/minute for 15 minutes at room temperature.

As a measure of cell proliferation, the protein content of each tube is then determined by the method of Oyama and Eagle (18). Two-milliliter aliquots are removed from each tube and transferred to a second set of tubes. One milliliter of water and 3 ml of fresh Lowry's reagent are then added to each of these tubes. They are shaken and allowed to stand for 15 minutes, and 0.5 ml of diluted Folin-Ciocalteu's reagent is then added with an automatic pipettor as each tube is vortexed. After 30 minutes, the optical density of each tube is measured at 660 nm. The optical density is representative of the amount of cellular protein present at the end of the incubation period. The exact amount of protein present in each tube is calculated by comparison to a bovine serum albumin standard which is run with each protein assay.

The effect of the treatment on the proliferation of the KB cells (as measured by increase in cellular protein) may be expressed as either "percent of control growth" or "percent inhibition of control growth." The percent of control growth is arrived at by dividing the average protein content of the duplicate treated tubes (minus the protein content of tubes when initially seeded) by the average protein content of the appropriate control tubes (minus the initial protein content) and multiplying the result by 100. The average initial protein content per tube, as determined in 37 experiments on seeding 68,000 cells/tube (20 tubes/experiment), was 19.4 μg/tube. The percent inhibition is obtained by subtracting the "percent of control growth" from 100%. Because treatment with the S-9 fraction alone causes a slight inhibition of growth (0–14%), the protein content of those tubes treated with

test agent and S-9 fraction is compared to the protein content of controls treated with S-9 fraction but no test agent. The protein content of tubes treated with test agent but without S-9 fraction is compared to that of controls treated with medium only.

Additional Modifications

Modifications for investigation of cofactor requirements.—This experiment was performed as described above except that the cofactors and S-9 fraction were prepared as three separate solutions, one containing $NADP^+$ at 4 mg/ml, one containing glucose-6-phosphate at 30 mg/ml, and one which was a 1:5 dilution of the original S-9 preparation. These solutions were then added in appropriate amounts to obtain the desired final concentrations.

Modifications for investigation of the effect of duration of treatment and time of initiation of treatment.—These experiments were performed as described above except for the obvious changes in duration of treatment and time of initiation of treatment as described in the Results section.

RESULTS

The results of our investigation of the optimal cofactor requirements for detecting the cytotoxic activity of cyclophosphamide at a concentration of 50 μg/ml are shown in table 1. These results show that either 0.3 or 0.6 mM NADP would be acceptable for the activation of cyclophosphamide. The 0.6-mM NADP level was chosen. Although we had used 12.5 mM glucose-6-phosphate in most of our experiments, our evidence indicates that 25 mM glucose-6-phosphate was superior to 12.5 mM in the activation of cyclophosphamide. The former might therefore be more suitable for application of this system.

In the standard KB assay, the treatment chemical and the cells being treated are incubated together for 72 hours; at that time the cell sheet is washed and a protein determination is performed. When an in vitro metabolic activation system is employed, the duration of treatment is at most a few hours, so the basic procedure must be modified. If the treatment is performed too soon after the cell attachment occurs, the recovery of treated cells can be very small and the protein measurements would not yield significant results. Alternatively, if treatment is initiated too close to the end of the 72-hour incubation, the difference in the total protein accumulated in the treated tubes compared to the control tubes will be small, because most of the cell proliferation will have occurred prior to the initiation of the treatment. To establish a treatment initiation time, we used a treatment incubation interval of 3 hours. Treatment was initiated with 50 μg/ml of cyclophosphamide at either 24 or 48 hours after inoculating cells into the tubes. As can be seen in table 2, the percent of control cell growth is definitely a function of the time of treatment. When KB cells were treated with S-9 fraction alone 24 hours after seeding, the amount of cellular protein accumulated at 72 hours was somewhat less (94%, 89%, 88%; average = 90% of control growth) than when treatment was initiated at 48 hours after seeding (93%, 86%, 100%, 94%, 100%, 88%, 95%; average = 94% of control growth). Our recommendation, based on these observations, is that treatments be initiated at either 36 or 48 hours after inoculation of the cells into tubes. The 48-hour treatment time has proven more convenient.

As stated above, the in vitro metabolic activation system is typically employed for short-term incubations; such systems are known to lose their metabolic capability after longer incubation times. Furthermore, as indicated above, the presence of the S-9 activation system itself can reduce the protein accumulation after additional incubation as compared to medium controls. Table 3 shows the effect of varying the duration of treatment, with and without a metabolic activation system present. The cells

TABLE 1.—Selection of optimum cofactor concentrations for metabolic activation of 50 μg/ml of cyclophosphamide

NADP concentration (mM)	Glucose-6-phosphate concentration (mM)	$MgCl_2$ concentration (mM)	% of control growth*
0.3	12.5	0.98	47
0.6	12.5	0.98	50
1.2	12.5	0.98	60
0.6	6.2	0.98	47
0.6	12.5	0.98	50
0.6	25.0	0.98	18
0.6	12.5	0.98	50
0.6	12.5	2.00	44
0.6	12.5	4.00	47

*Cell growth in the S-9 controls was 88% of that in the medium controls in this experiment.

TABLE 2.—Effect of varying time at which 3-hr treatment is initiated on cell growth measured at 72 hrs

Test agent (μg/ml)	Time after seeding (hrs)	% of control growth*	
		With activation	Without activation
Cyclophosphamide (50)	24	34	100
	48	64	97

*Cell growth in the S-9 controls was 94% of that in the medium controls when treatment was initiated 24 hrs after seeding and 97% of that in the medium controls when treatment was initiated 48 hrs after seeding.

TABLE 3.—Effect of varying length of treatment interval on cell growth measured at 72 hrs (treatment initiated 48 hrs after seeding)

Test agent (μg/ml)	Length of treatment (hrs)	% of control growth*	
		With activation	Without activation
Cyclophosphamide (250)	½	103	
	1	73	
	2	16	97
	3	19	100
DTIC (500)	1	113	
	2	93	100
	3	68	97

*Cell growth in the S-9 controls was 91% of that in the medium controls with the 2-hr treatment and 94% of that in the medium controls with the 3-hr treatment.

were treated either with 250 μg/ml of cyclophosphamide or 500 μg/ml of DTIC 48 hours after seeding the cells into tubes. In the case of cyclophosphamide, maximal inhibition of cell growth with an added metabolic activation system present occurred at 2 hours, while in the case of DTIC, the greatest percent inhibition achieved was at 3 hours. A treatment time of at least 3 hours is therefore recommended.

The amount of S-9 present in the reaction mixture can also affect the outcome of these treatments. Three different S-9 preparations were employed in our investigation of this parameter (table 4). One was an Aroclor 1254-induced mouse liver S-9 preparation which was prepared in our laboratory by the method of Ames et al (16); the second and third were Aroclor 1254-induced rat liver S-9 preparations, also prepared by this method and obtained from Litton Bionetics, Inc. At the concentrations of S-9 protein employed, the toxicity resulting from treatment for 3 hours with two rat liver preparations with 50 μg/ml of cyclophosphamide present appeared much greater than that resulting from treatment for 2 hours with the mouse liver preparation with 500 μg/ml of cyclophosphamide present. In all three instances, an optimum concentration range of the S-9 preparations was indicated. In figure 1, the concentration dependence of the Aroclor 1254-induced rat liver S-9 preparation was more closely examined during treatment of KB cells with three different chemicals; these included cyclophosphamide (50 μg/ml), DTIC (250 μg/ml), and 2-amino-1,3,4-thiadiazole (250 μg/ml). For both cyclophosphamide and DTIC, the S-9 concentration employed in our standard modified procedure is in the optimal range. The ability of the S-9 preparation at any of the tested amounts to activate 2-amino-1,3,4-thiadiazole at the concentration of 250 μg/ml appears questionable. In five different experiments, after treatment with 50 μg/ml of cyclophosphamide in the presence of 1.9 mg/ml of S-9 protein, the percent inhibition

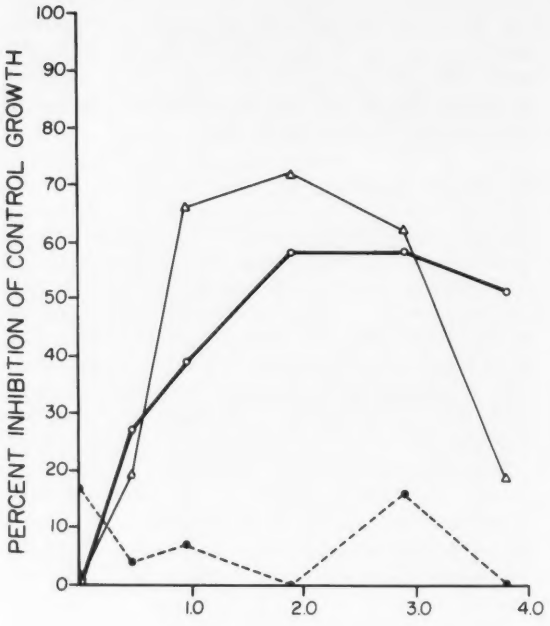

FIGURE 1.—Effect of varying final S-9 concentration (mg/ml) on the inhibition of KB cell growth when treated with 50 μg/ml of cyclophosphamide (△——△), 250 μg/ml of DTIC (○——○), and 250 μg/ml of 2-amino-1,3,4-thiadiazole (●---●). The S-9 fraction was prepared from Aroclor 1254-induced Sprague-Dawley rat liver by the method of Ames et al (16).

was 36% for one lot of S-9, 28% for a second lot, and 51%, 42%, and 74% for a third lot. The highest inhibition using the third lot was obtained 3½ months after the first value was obtained, with the S-9 being stored at –80°C during this time.

In table 5, we summarize data from several experiments in which we use the modified KB assay to examine the effect of the in vitro metabolic activation system on cell growth inhibition by known chemotherapeutic agents. Cyclophosphamide, DTIC, hexamethylmelamine, and pentamethylmelamine show activity only with the S-9 fraction present. The observed toxicity is dependent on the initial concentration of the agent. The presence of an induced S-9 preparation does not increase the observed activity of either melphalan or chlorambucil, neither of which is expected to require activation; in fact, the presence of the S-9 appears to interfere with the action of melphalan. Although 2-amino-1,3,4-thiadiazole is reported to require metabolic activation, we were unable to demonstrate inhibitory activity in our assay. This is not surprising, however, since this chemical apparently functions as an antimetabolite. It might be expected that the removal of any metabolic products after only 3 hours

TABLE 4.—Effect of S-9 concentration (mg/ml) on cyclophosphamide-induced cytotoxicity

S-9 preparation	Cyclophosphamide concentration (μg/ml)	S-9 fraction concentration (mg/ml)	% inhibition of control growth
IRL-65*	50	0.95	66
		1.9	28
		3.8	25
IRL-105†	50	0.48	19
		0.96	66
		1.9	74
		2.9	62
		3.8	19
MLM‡	500	0.75	6
		1.5	49
		3.0	0

*Prepared by Litton Bionetics, Inc, from Aroclor 1254-induced male Sprague-Dawley rat liver by the method of Ames et al (16) (lot No. IRL-65). Treatment time, 3 hrs; S-9 controls 93% of medium controls at 1.9 mg/ml of S-9.

†Prepared by Litton Bionetics, Inc, from Aroclor 1254-induced male Sprague-Dawley rat liver by the method of Ames et al (16) (lot No. IRL-105). Treatment time, 3 hrs; S-9 controls 86% of medium controls at 1.9 mg/ml of S-9 and 100% of medium controls at 3.8 mg/ml of S-9.

‡Prepared at the Southwest Foundation for Research and Education from Aroclor 1254-induced male $CD2F_1$ mouse liver by the method of Ames et al (16). Treatment time, 2 hrs; S-9 controls 100% of medium controls at 3.0 mg/ml of S-9.

of treatment would prevent its activity from being expressed. For example, treatment of KB cells for 2 hours with methotrexate, an antimetabolite not requiring metabolic activation, over a wide concentration range has no effect on the cloning efficiency of KB cells; when the treatment interval is increased to 20 hours, cell death does result (unpublished observation). Procarbazine was not active against KB cells either in the presence or absence of S-9.

We then examined selected materials known to be moderately active in vivo, but which we demonstrated to be inactive in vitro against KB, L1210, and P388 cells (unpublished observations). Two experiments were performed (table 6); in experiment 3, a concentration of 100 μg/ml of each chemical was used while in experiment 4, the chemicals were re-tested at a higher concentration (250 μg/ml). No detectable activation resulted. Three of the materials tested, NSC-265474 at 250 μg/ml and NSC-B827906 B003 and NSC-B833481 at both 100 and 250 μg/ml, did show activity without the S-9 present; in each instance, the addition of the S-9 eliminated detection of this activity. The latter effect could be artifactual, or could be due to a metabolic detoxification mechanism.

CONCLUSIONS

An in vitro metabolic activation system can be successfully used to demonstrate the growth inhibitory ac-

TABLE 5.—Activity of known antineoplastic agents against KB cell growth tested in vitro with metabolic activation

Material (NSC No.)	Concentration (μg/ml)	Experiment No.	% of control growth*	
			With activation	Without activation
Cyclophosphamide (26271)	50	3	49	
	100	3	2	103
DTIC (45388)	100	3	74	
	500	3	44	100
2-Amino-1,3,4-thiadiazole (4728)	100	5	95	105
	250	4	100	83
Hexamethylmelamine (13878)	100	4	100	
	250	4	69	106
Pentamethylmelamine (118742)	100	4	14	
	250	4	0	96
Melphalan (8806)	100	2	8	0
Chlorambucil (3088)	100	2	14	21
Procarbazine (77213)	500	1	111	100

*Cell growth in S-9 controls was the following % of that in medium controls: experiment 1, 94%; experiment 2, 89%; experiment 3, 100%; experiment 4, 88%; and experiment 5, 95%.

TABLE 6.—Activity of selected in vivo active materials against KB cell growth tested in vitro with metabolic activation

Material* (NSC No.)	Concentration (μg/ml)	Experiment No.	% of control growth†	
			With activation	Without activation
225325	100	3	97	115
	250	4	88	86
170347	100	3	88	106
	250	4	116	106
234764	100	3	82	100
	250	4	69	70
262666	100	3	88	109
	250	4	100	110
265474	100	3	62	76
	250	4	62	19
B819244 B002	100	3	88	88
	250	4	108	100
B827848 B003	100	3	82	85
	250	4	81	83
B827906 B003	100	3	70	8
	250	4	69	6
B831270	100	3	91	88
	250	4	112	75
B833481	100	3	94	79
	250	4	84	0

*For the natural products (B prefix), when no B00 number is listed, it is the 1st crude extract; B002 = the 2nd crude extract and B003 = the 3rd time a crude extract was prepared. NSC-225325, graft copolymer (sodium salt) of wheat starch with acrylic acid + a small amount of acrylamide; NSC-170347, 2,5-furandione, polymer with 1,1′-oxybis (ethene); NSC-234764, 1,3-benzenediol, dicarbamate; NSC-262666, 1,4-diazoniabicyclo [2.2.1] heptane, 1,4-bis (2-chloroethyl)-, (*Z*)-2-butenedioate (1:2); NSC-265474, phosphonium, [2-(1,3-dihydro-5-methyl-1,3-dioxo-2*H*-isoindol-2-yl)ethyl]triphenyl-, bromide; NSC-B819244, ethyl alcohol extract of root of *Zamia-SP;* NSC-B827848, 95% ethyl alcohol extract of wood of stems, stem bark, stem, and leaf of *Avicennia marina*; NSC-B827906, methyl alcohol extract of wood of stems, stem bark, stem, and leaf of *CF. Schotia latifolia*; NSC-B831270, ethyl alcohol extract of seed of *Loranthus cordifolius*; NSC-B833481, methyl alcohol extract of stem bark of *Allophylus loretensis*.

†Cell growth in S-9 controls was the following % of that in medium controls: experiment 3, 100%; experiment 4, 86%.

tivity in vitro of chemotherapeutic agents known to require such activation in vivo. While this is of significance if one is attempting to investigate the mechanism of action of these compounds, it does not appear from our results that the general application of in vitro metabolic activation for use in the NCI drug screening program is advantageous; even when we increased the likelihood of detecting in vitro activity by choosing materials which were active in vivo, we were not able to detect any in vitro activity with the added metabolic activation system. In our opinion, the additional cost and operations required for performance of the modified assay are not warranted for large-scale screening. In the case of one chemical for which in vivo metabolic activation was indicated, ie, 2-amino-1,3,4-thiadiazole, we did not detect activation by the Aroclor 1254-induced rat liver S-9 preparation. The value of the modified KB assay as a bioassay for the active components of in vivo-active materials was not demonstrated in these experiments; however, individual materials of high interest (ie, high in vivo activity) could be tested in this system to determine if it might be able to detect their activity in vitro and thus speed the purification of the active components of these materials.

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Polyestradiol Phosphate: A Preliminary Evaluation of Its Effect on Breast Carcinoma[1]

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SUMMARY

The effect of polyestradiol phosphate (Estradurin), a long-acting estrogen preparation given im, was assessed in 24 elderly postmenopausal patients with stage II or III primary or recurrent breast carcinoma. Although the drug has been available for many years, there has been no report in the literature of its use in treating breast carcinoma. The results of this study show that a total of 17 of 24 (70.8%) patients had evidence of tumor regression lasting a minimum of 3 months, while in 14 (58.3%) patients regression was maintained for ⩾ 6 months. Seven patients had complete (100%) tumor regression. Side effects were almost nonexistent and the agent has been shown to be effective, with good patient acceptability and guaranteed administration. It could be considered as an alternative to oral estrogen therapy where patient compliance is poor and gastrointestinal side effects are severe enough that patients often request a change of treatment.

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The purpose of this study was to assess the efficacy of a long-acting im estrogen preparation, polyestradiol phosphate (PEP), in the treatment of breast carcinoma in elderly female patients. The preparation has been used with good effect in the treatment of prostatic neoplasms in males (1), where there is evidence that the survival rate is higher with PEP than with other endocrine therapies. There has been no report in the literature, however, of its use in breast cancer.

It was decided to administer PEP according to the regimen recommended for prostatic neoplasms (80 mg every 4 weeks by deep im injection for three doses) and then to reduce the dose to a maintenance level of 40 mg monthly for as long as the disease was in remission. PEP was given to all elderly patients referred to our department with surgical stage II or III breast carcinoma determined by histologic and clinical examination. Basic investigations included full blood cell count, liver function tests, and either a bone scan or a limited skeletal survey (chest, skull, whole spine, and pelvis). Any patient with a fungating or painful lesion requiring more rapid treatment with irradiation for symptomatic relief and patients with severe congestive cardiac failure for whom estrogens were contraindicated for various reasons were excluded.

All of the patients in this study were considered to be unsuitable for surgery, either because of their age and/or infirmity or because they had refused an operation. Twelve patients had received irradiation to the primary lesion (with complete response) between 6 months and 7 years previously and were thus being treated for a recurrence at the primary site. None of these patients had received previous cytotoxic or hormonal therapies. Disease characteristics at the time of patient presentation are shown in table 1.

PHARMACOLOGY

Im PEP is a long-acting estrogen preparation containing a water-soluble high molecular weight polyester of

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TABLE 1.—Disease characteristics at the time of patient presentation

Characteristic	No. of patients (total = 24)
Overall breast lump size = 4–8 cm	19 (79.0%)
Multiple dermal nodules (all recurrent)	3 (12.5%)
Fungating tumor	1 (4.2%)
Ulcerating tumor (6 cm in diameter)	1 (4.2%)
Tumor in association with axillary nodes	3 (12.5%)

estradiol, 17β-phosphoric acid, which acts primarily as a long-acting form of estradiol (2). The structural formula is shown in figure 1. It is not a true depot preparation because it disappears from the injection site within 48 hours, when it is then found to be stored in combination with proteins in the reticuloendothelial system from which it is slowly released in a predictable manner (3). In the Allen-Doisy test on mice (3), it has been shown that 20 μg of PEP will produce an estrogenic effect lasting at least 14 days, while the effect produced by the same quantity of estradiol benzoate or ethinylestradiol lasts only 4 days. It has been shown that constant blood levels of estrone, estradiol, and estriol were produced for 30 days in a 47-year-old female after a single injection of 80 mg of PEP (4).

Zurcher (5) has shown that a single injection of 40 or 80 mg given to oophorectomized women produced a rise in the blood levels as well as constant levels of urinary excretion of estrone, estradiol, and estriol lasting 4–6 weeks, which is equivalent to the levels in a normal menstruating female. This prolonged activity has been attributed to the inhibiting effect on phosphatase leading to a slow breakdown of the polymer in vivo.

MODE OF ACTION

There is still some doubt about the exact mechanism by which estrogens produce tumor regression in breast cancer. Pituitary suppression is one obvious effect, since stilbestrol is known to reduce the excretion of 17-ketosteroids, androsterone, and ethiocholanolone. Emmerson et al (6) proposed that estrogens stimulate hyperplasia of the breast stroma with enhancement of the normal tissue healing mechanism, and they suggested that the resulting influx of lymphocytes and plasma cells could produce an immunologic response. Another hypothesis (7) suggested that the effect of estrogen therapy depends on the absolute and relative concentrations of prolactin and estrogen actively available at the tumor site, with the differences in site sensitivity dependent on tumoral factors, such as the levels of estradiol and prolactin binding receptors in the tumor.

PEP appears to have a different mode of action on the pituitary gland in that there is little or no reduction of the levels of 17-ketosteroids, androsterone, and ethiocholanolone (1). It has also been shown (2) to be an inhibitor of several enzyme systems, such as acid and alkaline phosphatase and hyaluronidase. Others (1) have suggested that it may exert a direct cytotoxic effect on cancer cells. If any of these hypotheses are correct, it would appear that the unique method of storage and the subsequent release mechanism of PEP which maintains a constant elevated blood estrogen level can only be advantageous in the treatment of breast cancer.

PATIENT ASSESSMENT

The size of the primary tumor and the number, location, and dimensions of any metastases were noted on presentation; the patients' cardiac state was also re-

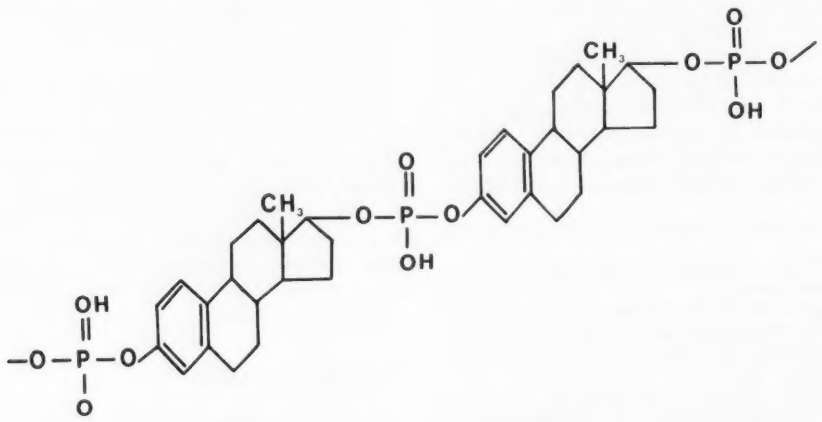

FIGURE 1.—Structural formula of PEP.

corded. The pulse, blood pressure, jugular venous pulse, and any evidence of ankle edema were recorded. Every 4 weeks, immediately preceding the next injection, the same parameters were measured. Possible side effects such as nausea, vomiting, vaginal bleeding, or pain at the injection site were recorded. Any patient who failed to show a response at 3 months was changed to another therapy.

CRITERIA OF RESPONSE

Many different criteria of response have been used to describe the results of the various therapies for breast carcinoma. In this study we have conformed to the system suggested by the UICC (8), which is as follows: *complete response*—disappearance (100%) of all known disease; *partial response*—> 50% decrease in measurable lesions; *no change*—measurable lesions unchanged, ie, < 50% decrease or up to 25% increase in the size of measurable lesions; and *progressive disease*—progression of some or all lesions and/or appearance of new lesions. Our goal was to obtain regression according to the above criteria lasting a minimum of 3 months, which was achieved in 17 (70.8%) of the 24 patients treated with PEP.

RESULTS

All of the 24 patients entered in this series received a minimum of three monthly im injections of PEP and were fully re-assessed prior to the administration of the fourth injection which was to be reduced to 40 mg. At this time, only three patients had failed to respond in that the bidimensional measurements of their tumors had increased by up to 25%. Although there did not appear to be any evidence of accelerated growth, hormone therapy was terminated in these patients and alternative treatment was given (a course of irradiation in two patients and surgery in the third patient).

The overall response according to the criteria was maintained for a minimum of 3 months in 17 patients (70.8%). The results are summarized in table 2. Complete regression was noted in five patients (20.8%) and partial regression in 12 (50%). It should be noted that four of the patients said to have shown no response did have tumor shrinkage of between 25% and 50% which was maintained for the 3-month period. We continued to administer PEP to this group with the hope that their responses would improve but mainly because their tumors were under some degree of control and the patients were content with the situation. We were then able to assess 16 patients who had responded for a minimum of 6 months; at this time there were seven patients in complete remission and seven in partial remission while two still had no change (their tumors had remained stable).

Eight patients were lost to the study. Three who had tumor enlargement as mentioned above had an alternative treatment. Two patients died while their tumors were under partial control. Although postmortem examinations were not performed, no new metastases had developed and it was not thought that PEP had contributed to their deaths. Two patients developed vaginal bleeding and requested a change in treatment, and the eighth patient had had a partial response for > 3 months but developed a recurrence of her tumor which did not respond to alternative measures and she died with distant metastases. To date, eight patients have shown a continuous response lasting 1 year, three of whom are in complete response and five of whom are in partial remission.

TABLE 2.—Response to PEP

Response	No. of patients with a minimum duration of response of—	
	3 mos	6 mos
Complete (100%)	5/24 (20.8%)	7/24 (29.16%)
Partial (> 50%)	12/24 (50%)	7/24 (29.16%)
No change	7/24 (29.16%)	2/24 (8.33%)

There appears to be no difference in the response of the previously irradiated group of patients who were treated for recurrent tumors; however, this series is probably too small for any significant difference to manifest itself.

In 18 patients (75%) some degree of tumor regression was noted at the first followup visit, 4 weeks after the initial injection of PEP. The maximum response was seen in the majority of patients at 3 months, although in two patients it took longer than this to achieve complete remission. These results are in agreement with published reports on the length of time required for oral estrogens to be effective. One series states that 90% of patients had a response within 8 weeks but complete remission took several months (9).

SIDE EFFECTS

The side effects of oral estrogens are well-documented (10,11); they include anorexia, nausea, vomiting, pigmentation of the areola or axilla, engorgement of the breast, softening of the skin, diarrhea, and fluid retention. Estrogens are also known to precipitate congestive cardiac failure. There has been no evidence in the literature of any toxic effects resulting from the administration of PEP, even when ten times (400 mg) the therapeutic dose has been given monthly for 1 year (12), nor has there been any evidence of liver damage.

In this series of patients the incidence of side effects was extremely low; none of the patients had any nausea, vomiting, or dyspeptic symptoms, nor has there been any report in the literature of these effects. We have seen no

evidence of any cardiovascular problems, although four patients in this series were already receiving treatment with diuretics and digoxin for mild cardiac failure when im estrogen therapy was instituted. Since none of these patients had exacerbation of their symptoms, these findings agree with those from another series (1) on the long-term administration of PEP to elderly males with prostatic carcinoma.

Vaginal bleeding due to endometrial hyperplasia is a common problem in women receiving estrogen therapy, with some 12% of all patients developing this symptom (13). This has been the only adverse effect we have noted, with bleeding occurring in only six patients. In three cases bleeding was severe in that it was continuous and required a change of treatment. In two other cases there was only intermittent spotting, lasting 3 and 7 days respectively. The sixth patient was found to have a uterine polyp and had no further problems after this was excised. Bleeding started in all cases before the fifth injection after the dose had been reduced to 40 mg. Thus, it may be that maintaining a constant dose level of either 80 or 40 mg will reduce the incidence of this problem.

DISCUSSION

The role of estrogens in the management of breast carcinoma in the postmenopausal patient has been well-established for the last 30 years. One of the earliest reported series (14) describes the effects of a number of synthetic estrogens on 40 women with advanced breast carcinoma; 14 of the patients received stilbestrol which produced tumor regression in 35% of the patients. In the same year, at a meeting of the Radiology Section of the Royal Society of Medicine (15), the speakers described the effect of estrogens on a combined total of 121 patients, of whom 5% had complete tumor regression.

Estrogen therapy has proved over the years to be one of the simplest, most effective methods of endocrine therapy for older patients with breast malignancy. The remission rate (10) has remained at 50% for patients with inoperable tumors, although the effect of a given dose of estrogen is well-known to be age-dependent. In one series (7) an overall regression rate of 35% was increased to 59% when only patients $\geq$ 80 years of age were considered. In our series the age range of the patients was 62–97 (average, 75.2 years), with only four patients $\geq$ 80 years and three $<$ 70 years. It is an established fact that one would expect the type of patient described in this study to respond well to oral estrogens since they are all elderly with local disease. If PEP is an effective agent then the response must be as good, if not better, when used for the treatment of this group of patients.

The only remaining controversy about the application of estrogens concerns the dose to be administered, but even here it has been shown that response rates are the same for both low-dose schedules of 1.5 mg of stilbestrol daily and high-dose schedules of 15 mg of stilbestrol daily (7).

Elderly patients are notoriously unreliable at taking tablets regularly, especially if they have been prescribed multiple preparations for a range of illnesses. Patients may often be confused, senile, or obstinate, and side effects such as nausea, vomiting, dyspeptic symptoms, or vaginal bleeding may also reduce the patients' enthusiasm for prolonged oral therapy. One study (16) on elderly males taking oral estrogen for the treatment of prostatic cancer revealed that 30% of the patients stopped taking their tablets when their symptoms improved. Legha et al (17), in their review of the hormonal treatment of breast carcinomas, state that the noncompliance rate is 10% with oral estrogens due to gastrointestinal side effects and fluid retention. It is thought (18) that there is a greater tendency to discontinue oral estrogen therapy prematurely with increasing age due to severe side effects such as vomiting or cardiac embarrassment.

The younger patient can be encouraged to continue treatment and accept mild symptoms, such as loss of appetite, ankle edema, or stress incontinence, whereas the elderly patient usually insists on a change of treatment with a consequent loss of the very high likelihood of tumor regression for this age group. In a series of 170 patients $\geq$ 70 years of age, almost 50% discontinued oral estrogen therapy before completing 1 month of treatment (18). Thus, the advantages of an effective regular monthly im preparation are obvious. Not only is there guaranteed administration of the agent, but the incidence or severity of side effects may be reduced.

CONCLUSION

The results of this study are encouraging in revealing a large proportion of patients (70.8%) responding to a long-acting im estrogen preparation. While it is ideal to produce complete regression of any tumor, partial regression or merely disease control must be considered a satisfactory result in elderly patients, especially if their quality of life improves or if they have other medical problems. This preliminary study indicates that im PEP appears to be an effective alternative to oral estrogens, with the added advantages of guaranteed administration, maintenance of adequate estrogen levels, and excellent patient acceptability due to a lower incidence of side effects. When estrogen therapy is indicated for the treatment of carcinoma of the breast, it is suggested that an im preparation be used and maintained for as long as the tumor shows a response, since the maintenance of constant high estrogen levels for prolonged periods might be advantageous for this type of patient.

Continued evaluation of this preparation in a larger series of patients will determine its proper role in the

management of breast carcinoma in the postmenopausal patient.

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1-Year Followup of Cardiac Status After Adriamycin Therapy[1]

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SUMMARY

To study the natural history of the alterations in cardiac function produced by Adriamycin therapy, serial M-mode echocardiograms, electrocardiograms, and systolic time intervals were obtained in 15 patients prior to Adriamycin therapy and at 3-month intervals during and 1 year after cessation of therapy. The echocardiographic E-point septal separation increased from a median 0 dose value of 0.40 cm to 0.70 cm ($P < 0.003$) at the time of the maximum dose of Adriamycin. The electrocardiographic frontal plane voltage decreased 13% ($P < 0.008$) and the pre-ejection period/left ventricular ejection time ratio increased from a median value of 0.37 to 0.40 ($P < 0.01$) during this same period. During the first year after Adriamycin was discontinued, no significant change was noted in any of these values. Thus, as a group, the slight deterioration of ventricular function that occurred during Adriamycin therapy remained unimproved during the 1-year followup period.

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The anthracycline antibiotic, Adriamycin, has become one of the most commonly used anticancer agents in the world (1). A serious and unwanted side effect of a high cumulative dose of Adriamycin is the development of cardiomyopathy that may lead to congestive heart failure (CHF) and may even be fatal (1–5). Fortunately, this complication is not common when the total cumulative dose of Adriamycin is limited (1,2). However, most patients receiving Adriamycin show some deterioration in cardiac function as documented by noninvasive techniques, even when there is no overt clinical evidence of CHF (6–8). There are little data available concerning the natural history of these alterations in cardiac function after Adriamycin is discontinued. Fifteen patients who completed a full course of Adriamycin therapy had serial noninvasive evaluation of their cardiac status for 1 year after completion of therapy. These patients form the basis of this report.

METHODS

A prospective study of the sensitivity and specificity of various noninvasive techniques in assessing ventricular function in patients receiving Adriamycin was begun at the University of Arizona Health Sciences Center in 1974. Fifteen patients who have participated in this ongoing prospective study have completed the prescribed course and have been followed for 12 months after their last dose of Adriamycin therapy. Table 1 shows the clinical information on these patients. Each patient had an echocardiogram, phonocardiogram, systolic time intervals, and an electrocardiogram (ECG) prior to beginning Adriamycin therapy and at 3-month intervals during and after chemotherapy.

M-mode echocardiograms were obtained with an echocardiographic unit (Ekoline-21) having a repetition rate of 1000 pulses/second utilizing a 2.25-MHz transducer with a 13-mm diameter crystal. The echocardiograms were recorded on a Honeywell-1856 strip chart recorder with simultaneous ECG reference lead. The patients were examined in the supine or the slight left-lateral

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TABLE 1.—Clinical data

Patient No.	Age	Type of cancer	Potential risk factors for cardiotoxicity	Cumulative dose (mg/m²)	Clinical course*
1	69	Breast	Chest radiation†	401	Developed CHF
2	51	Breast	None	374	NS
3	63	Ovarian	None	387	NS
4	18	Thyroid	None	359	NS
5	71	Lung	Age‡	476	Developed dyspnea; no clinical CHF
6	59	Breast	None	460	Died; sepsis
7	57	Breast	None	429	NS
8	74	Ovarian	Age‡	462	Developed dyspnea; no clinical CHF
9	67	Breast	None	417	NS
10	55	Ovarian	None	415	NS
11	57	Lymphoma	None	395	NS
12	56	Breast	None	454	NS
13	37	Adenocarcinoma (primary unknown)	None	511	NS
14	54	Breast	None	420	NS
15	57	Lymphoma	None	550	Developed CHF

*NS = no symptoms.
†Previous thoracic radiation.
‡>70 yrs.

position with the transducer at the left sternal border where the mitral valve echoes were seen best. The cardiac echoes were continuously recorded as the transducer was tilted slowly through an arc with the ultrasonic beam traveling from the area of the aortic valve to the left ventricle. Left ventricular internal dimension measurements were determined as described previously (8). The E-point to septal separation (EPSS) was measured as the shortest distance from the E-point of the anterior mitral valve echo to a horizontal line drawn from the most posterior point of the left side of the interventricular septum recorded during systole (fig 1) (8,9).

Standard 12-lead ECGs were recorded utilizing either a Marquette series-2000 or a Hewlett-Packard-1517A three-channel ECG machine. Frontal plane ECG voltage was calculated according to the method of Minow et al (6). Systolic time intervals were recorded by the method of Ewy et al (8) and Weissler et al (10).

Statistical comparisons between median values obtained (*a*) prior to the initiation of Adriamycin therapy, (*b*) at the time of the maximum Adriamycin dose, and (*c*) at 6 months and 1 year after Adriamycin treatment were made using the Wilcoxon-signed rank test (11).

RESULTS

The clinical data are summarized in table 1. Three patients had risk factors for developing Adriamycin cardiotoxicity (12,13). One patient (No. 1) had received prior thoracic radiation therapy for breast cancer. This was one of the two patients in the group to develop clinical evidence of CHF. Cardiac decompensation in this patient appeared after receiving a cumulative dose of 401 mg/m² of Adriamycin. Two patients were > 70 years old when Adriamycin therapy was initiated. Both of these patients have had symptoms of shortness of breath but have not had objective evidence of CHF.

One other patient in the group (No. 15) developed CHF. This patient had no known risk factors for the development of Adriamycin cardiotoxicity and appeared to have tolerated a maximum dose of 550 mg/m². He did well until 1 year after Adriamycin therapy was discontinued when clinical CHF became evident.

Two patients had been treated for hypertension with antihypertensive regimens including salt restriction and diuretics. Neither patient has had signs or symptoms of CHF.

Only one patient died during the followup period and

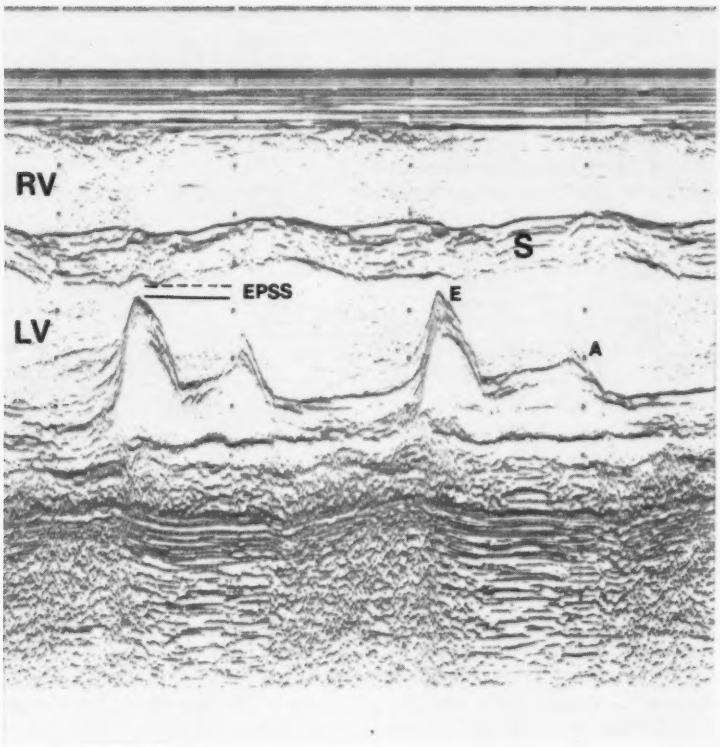

FIGURE 1.—M-mode echocardiogram at the mitral valve level. The EPSS is measured from the E-point of the anterior mitral valve leaflet to a horizontal line drawn for the most posterior point of the left side of the interventricular septum (S) recorded during systole. A = A-point of the anterior mitral valve leaflet; E = E-point of the anterior mitral valve leaflet; LV = left ventricle; RV = right ventricle.

his death was related to tumor and sepsis. This patient did not have clinical evidence of CHF; however, an autopsy was not performed.

Echocardiograms

Technically adequate serial M-mode echocardiograms were available in ten patients. None of the patients had pericardial effusions detected by echocardiography during the study period. The fractional shortening in left ventricular minor axis dimension (left ventricular diastolic dimension–left ventricular systolic dimension/left ventricular diastolic dimension) had decreased from a median value of 0.36 before treatment to 0.33 at the time of the maximum cumulative dose of Adriamycin (fig 2). This decrease was not statistically significant at the $P < 0.05$ level. During the followup period, the fractional shortening continued to fall so that at the 1-year followup the median value was 0.29, significantly decreased ($P < 0.01$) from the value determined prior to the initiation of therapy and from the value at the time of the maximum cumulative dose of Adriamycin. As shown in figure 2, the individual patient responses were quite variable.

The fractional shortening of the left ventricular minor axis dimension of patient 15, the only patient from this group to develop clinical CHF, decreased from a value of 0.38 before treatment to 0.17 at the time of the maximum cumulative dose of Adriamycin. Clinical CHF did not become apparent until 1 year after the maximum dose, at which time the fractional shortening value was 0.10.

Serial EPSS values for each patient are shown in figure 3. For the group, the EPSS was significantly increased at the time of the maximum cumulative dose of Adriamycin (median value, 0.70 cm) when compared to the pretreatment EPSS (median value, 0.40 cm) ($P < 0.03$). During the followup period, there was a tendency for the EPSS to return towards the pretreatment level; however, no statistical difference was found between the EPSS measured at the 6-month or 1-year followup and that measured at the time of the maximum cumulative

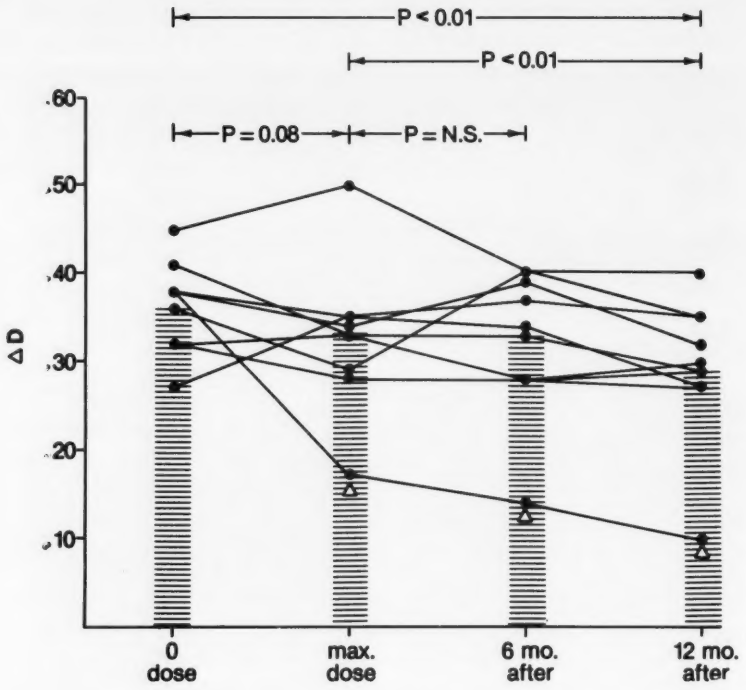

FIGURE 2.—Echocardiographic fractional shortening of the left ventricular minor axis diameter (ΔD) prior to Adriamycin (0 dose), at the time of maximum dose (max. dose), and at 6 and 12 mos after Adriamycin was discontinued in 10 patients. Lines connect serial measurements for the same patient. Δ = values for patient 15, the only patient in the group to develop clinical CHF. Bars = median value for the group at each time period.

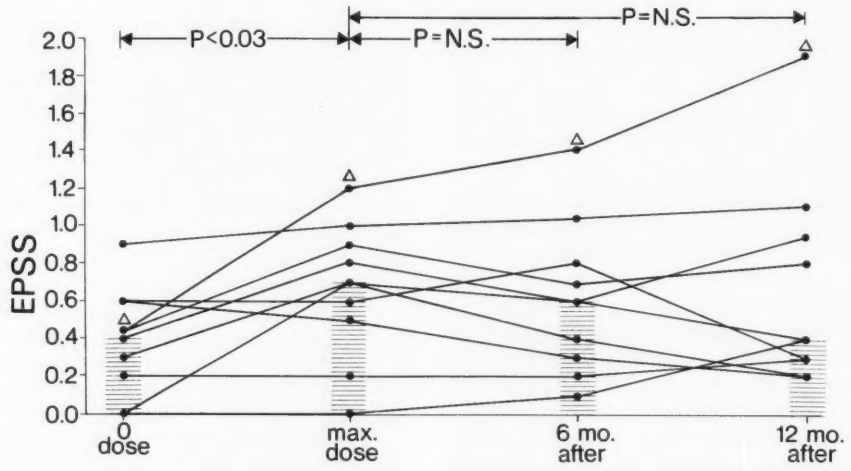

FIGURE 3.—Echocardiographic EPSS prior to Adriamycin (0 dose), at the time of maximum dose (max. dose), and at 6 and 12 mos after Adriamycin was discontinued in 10 patients. Lines connect serial measurements for the same patient. Δ= values for patient 15, the only patient in the group to develop clinical CHF. Bars = median value for the group at each time period.

dose of Adriamycin. The individual patient responses were variable, with two patients showing an initial rise in EPSS followed by a fall to pretreatment levels at the 1-year followup, four patients showing an initial rise in EPSS which remained increased through the followup period, and four patients showing little change in EPSS during the study period.

The EPSS of patient 15 increased from a value of 0.40 cm before treatment to 1.2 cm at the time of the maximum cumulative dose of Adriamycin. At the time that clinical CHF became apparent, the EPSS value had increased to 1.9 cm.

Electrocardiograms

Serial ECGs were available in 13 of 14 patients. One patient (No. 3) did not have an ECG at the time of the maximum cumulative dose and therefore was not included in this analysis. Six patients had 12-lead ECGs interpreted as normal throughout the study period. Five patients had nonspecific ST-T changes, three had abnormal left axis, one had left atrial abnormality, two had premature atrial beats, and one had premature ventricular beats on the standard 12-lead ECG. Patient 1 had a left atrial abnormality develop on her ECG. However, this did not occur until 6 months after the clinical diagnosis of CHF was established. Patient 15 developed left axis deviation and nonspecific ST-T changes at the time of the maximum cumulative dose of Adriamycin. However, 1 year after the maximum cumulative dose when clinical CHF was present, the ECG was unchanged from that obtained at the time of the maximum cumulative dose.

The percent change in frontal plane voltage on the serial ECG studies is shown in figure 4. The frontal plane ECG voltage was significantly lower at the time of the maximum cumulative dose of Adriamycin when compared to the pretreatment frontal plane ECG voltage ($P < 0.008$). At 6-month and 1-year followups, no significant difference was noted between the percent change in ECG frontal plane voltage recorded at the time of the maximum cumulative dose and that recorded at the followup times. None of the four patients who showed a $> 30\%$ decrease in ECG frontal plane voltage had clinical evidence of CHF.

Systolic Time Intervals

Serial systolic time intervals were available for each patient in the study group. The ratio of pre-ejection period to left ventricular ejection time (PEP/LVET) is shown for each patient in figure 5. The PEP/LVET ratio showed a statistically significant increase from a median pretreatment level of 0.37 to a median level of 0.40 recorded at the time of the maximum cumulative dose ($P < 0.01$). At 6-month and 1-year followups, no significant difference was noted between the median PEP/LVET ratio recorded at the time of the maximum cumulative dose and that recorded at these followup times.

The PEP/LVET ratio of patient 1 had only increased from 0.42 to 0.45 at the time she developed clinical CHF. The PEP/LVET ratio increased to 0.57 6 months after the maximum cumulative dose at the time when the CHF was adequately controlled with digitalis and diuretics. At 12 months following the maximum cumulative dose, the PEP/LVET had returned to pretreatment levels. The PEP/LVET ratio in patient 15 increased from 0.39 to 0.45 at the time of the maximum cumulative dose of Adriamycin. The ratio increased to 0.59 1 year after the maximum cumulative dose when clinical CHF was present. No other patient in this group had clinical CHF although the PEP/LVET was above the upper limits of normal (0.44) or had increased > 0.1 from pretreatment values in three additional patients.

DISCUSSION

Adriamycin has been shown to produce myocardial cell degeneration in rabbits (14) and in man (13). These pathologic findings antedate any alteration in left ventricular function, and overt CHF appears only after a critical amount of myocardial degeneration has occurred (13,14).

Several studies using noninvasive techniques (6–8) have demonstrated alterations of cardiac function prior to the development of overt CHF following the administration of high cumulative doses of Adriamycin. However, it is not known whether these alterations in cardiac function progress, remain stable, or revert to baseline following cessation of Adriamycin therapy.

Jaenke (15) has shown that Adriamycin-associated myocardial degeneration in the rabbit may progress for months after the drug is withdrawn, and a similar process may occasionally occur in man (13,16). One patient from our group (No. 15) appears to have demonstrated this pattern, since clinical CHF did not become apparent until 1 year after Adriamycin was discontinued. However, Bristow et al (13) have shown that the myocardial degenerative changes demonstrated on right ventricular biopsy were similar in five patients studied at a mean of 14.6 months after cessation of Adriamycin therapy to those changes seen in a dose-matched control group biopsied 1 month following the last dose of Adriamycin. Although the pathologic lesion appeared to remain stable after cessation of Adriamycin therapy, the PEP/LVET ratio was significantly shorter in the subjects studied many months after cessation of Adriamycin therapy when compared to the dose-matched control group studied 1 month after the last dose of Adriamycin. This suggested that myocardial performance improved with time (13).

This study demonstrated that none of the noninvasive measurements of cardiac function that showed significant

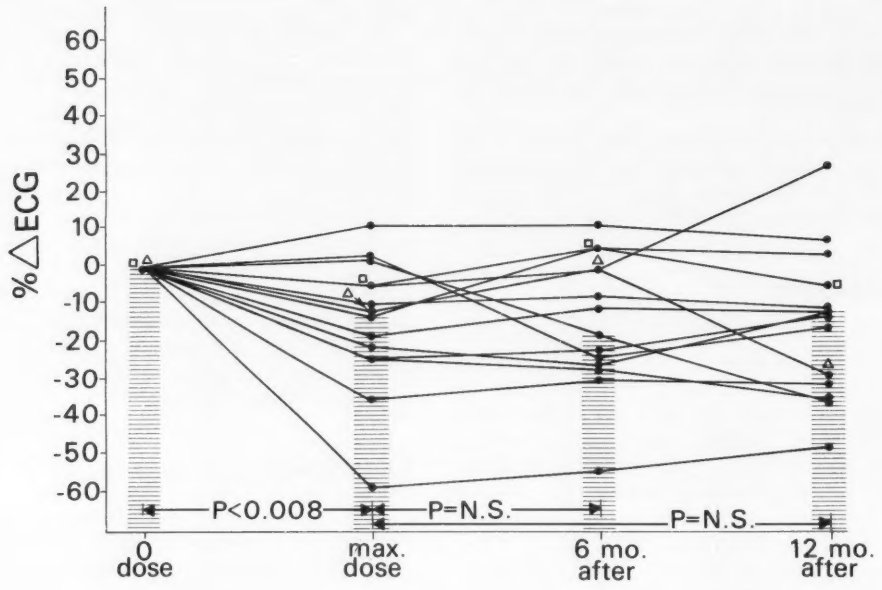

FIGURE 4.—% change in frontal plane ECG voltage (%△ECG) prior to Adriamycin (0 dose), at the time of maximum dose (max. dose), and at 6 and 12 mos after Adriamycin was discontinued in 14 patients. Lines connect serial measurements for the same patient. □ = values for patient 1 and △ = values for patient 15. Bars = median value for the group at each time period.

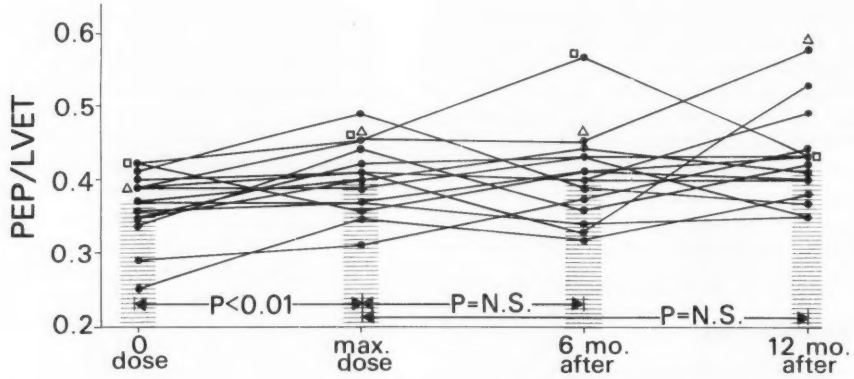

FIGURE 5.—PEP/LVET prior to Adriamycin (0 dose), at the time of maximum dose (max. dose), and at 6 and 12 mos after Adriamycin was discontinued in all patients. Lines connect serial measurements for the same patient. □ = values for patient 1 and △ = values for patient 15. Bars = median value for the group at each time period.

deterioration from pretreatment values to the time of the maximum cumulative dose showed any significant change towards pretreatment values during the 1-year followup period. In fact, the echocardiographic fractional shortening of the left ventricular minor axis diameter continued to decrease after cessation of Adriamycin therapy. This would suggest that, as a group, patients who demonstrate alterations of cardiac function due to Adriamycin therapy will continue to show these alterations for at least 1 year after the drug is stopped. However, individual patient variation is present.

We think the major reason for the discrepancy between our data and those reported by Bristow et al (13) is that in our study, serial examinations were performed on the same patient, allowing each patient to act as his own control. In their study, the patients studied many months after discontinuing Adriamycin therapy were compared to a control group matched only for total cumulative dose. Since the individual patient response to Adriamycin therapy is so variable and the number of patients reported is so small, the study and control group in their report may not have been comparable. Continued followup of large numbers of patients will be needed to resolve this question.

In the present study, two echocardiographic measurements of left ventricular function were analyzed: the fractional shortening of the left ventricular minor axis dimension and the mitral valve EPSS. Both measurements suggested a small deterioration in left ventricular function at the time of the maximum cumulative dose of Adriamycin with no significant improvement during the 1-year followup period. Both techniques showed the greatest change in the one patient from this group who developed clinical CHF. However, the maximum change in each measurement from this patient occurred between the start of Adriamycin therapy and the time of the maximum cumulative dose, while clinical CHF did not occur until 1 year after Adriamycin was discontinued.

The mitral valve EPSS has not been widely used for the evaluation of left ventricular function; however, previous studies (9) have shown good correlation between this measurement and angiographic and other echocardiographic measures of left ventricular function. Although the EPSS was not superior to any other noninvasive measure of ventricular function in the present study, it should be noted that the EPSS is a simple measurement to perform and may be obtained in adult patients who have technically inadequate echocardiograms for other measures of left ventricular function.

Recent studies (17,18) have suggested that radionuclide angiography may be more sensitive than other noninvasive techniques in detecting alterations in left ventricular function secondary to anthracycline therapy. Singer et al (17) reported that the radionuclide ejection fraction was depressed with high cumulative doses of Adriamycin or daunorubicin and that the ejection fraction improved in four patients 14–24 weeks after the anthracycline was terminated. Alexander et al (18) were able to identify all five patients from their group who developed CHF by demonstrating dramatic declines in left ventricular ejection fraction prior to clinical evidence of cardiac decompensation. The six patients who had their Adriamycin discontinued after demonstrating "moderate cardiotoxicity" did not develop clinical CHF. Followup evaluations, at $\leq$ 4 months after Adriamycin was discontinued, in 18 patients without clinical CHF demonstrated stable ventricular function with a mean increase in ejection fraction of 5%. Although the radionuclide techniques appear promising, larger studies with longer followup periods are needed before their true value can be determined.

The statistical analysis of our data was performed using the Wilcoxon-signed rank test (11). This statistical technique was chosen over the more familiar t test because the t test and related normal theory procedures assume independent, random samples from normally distributed populations having the same variance. The paired measurements in this study demonstrated a consistent lack of symmetry and equality of variation. Thus, the nonparametric Wilcoxon-signed rank test is more appropriately used to test whether the underlying distributions are identical.

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Enhancement by Thymidine of 5-Fluorouracil's Inhibition of Thymidylate Synthetase in Human Tumors [1,2]

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SUMMARY

The effects of thymidine (TdR) on the conversion of 5-fluorouracil (5-FU) to 5-fluoro-2′-deoxyuridine-5′-monophosphate and the resulting inhibition of thymidylate synthetase was determined in crude, human tissue extracts. TdR alone had no effect but it augmented the inhibition of thymidylate synthetase produced by 5-FU. TdR augmentation was probably mediated either by increasing 5-FU conversion to 5-fluoro-2′-deoxyuridine-5′-monophosphate or by inhibiting 5-FU catabolism.

[Cancer Treat Rep 63:1817–1820, 1979]

5-Fluorouracil (5-FU) was synthesized by Duschinsky et al (1) in 1957 and has been used since to treat human solid tumors. A response rate of about 20% is obtained when 5-FU is used to treat tumors such as breast and colonic carcinoma. However, drug administration is limited by host toxicity.

The mechanism of action of 5-FU is believed to be competitive inhibition of thymidylate synthetase by the 5-FU metabolite, 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP) (2,3). 5-FU activity requires conversion to FdUMP, a process requiring several enzymes (4–7). Thymidylate synthetase is the enzyme required for the addition of a CH_3 group at the 5 position of 2′-deoxyuridine-5′-monophosphate (dUMP) to form thymidine-5′-monophosphate (TMP). Inhibition of thymidylate synthetase results in decreased cellular incorporation of deoxyuridine and single carbon units into DNA-thymine. An unknown amount of 5-FU's action may be due to mechanisms such as its incorporation into RNA. An alternative pathway for synthesizing TMP utilizes thymidine (TdR) as a precursor and thymidine kinase as the enzyme. TdR administration, however, does not reverse 5-FU toxicity (8,9) which should happen if thymidylate synthetase inhibition were the principal site of 5-FU action. Paradoxically, several studies have shown that TdR administration augments rather than reverses the effect of 5-FU (10–14). This is believed to be due either to enhanced incorporation of 5-FU into RNA, enhanced conversion of 5-FU to FdUMP, or competition between 5-FU and TdR for the same catabolic enzymes. Concomitant 5-FU and TdR administration has been considered a means of enhancing the chemotherapeutic effectiveness of 5-FU (8,15). The crude tissue preparations used in the present study contained enzymes necessary for converting 5-FU to FdUMP and for catabolizing FdUMP. These activities were important in producing the overall inhibition of thymidylate synthetase measured in the study.

METHODS

Tissue Preparation

Tissue samples were obtained from specimens resected from patients undergoing surgery who gave informed consent. Tumor samples were selected from the viable-appearing margins of the tumors; samples of normal bowel consisted only of mucosa which had been freed from the underlying muscle with sharp dissection. Excised tissues were immediately stored in liquid nitrogen. Sample preparations started by pulverizing the specimens while they remained frozen. Two volumes of 0.01 M Tris buffer (pH 7.5) were added to the smashed, frozen tissue. More complete cell disruption was accomplished

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by ultrasonication with a Branson Sonifier at 90 watts for 30 seconds. The suspension was then centrifuged at 2000 × *g* for 30 minutes; the supernatant was saved and recentrifuged at 105,000 × *g* for 60 minutes. The protein content of the supernatant portion was determined (16), and aliquots of the supernatant were frozen and stored at 0°C. All tissue manipulations were done at 4°C and fresh aliquots of the original samples were used for each series of assays. Once-thawed tissue samples were discarded. Enzyme activity was unaffected by the addition of 1 mM dithioerythritol during tissue preparation and was unaffected by freezing and thawing twice.

Thymidylate Synthetase Assay

The activity of thymidylate synthetase was determined by measuring tritiated water formation from [5-^{3}H]-deoxyuridine-5′-monophosphate (^{3}H-dUMP) using a modification of the method of Roberts (17). Twenty-five to 100 µl of prepared tissue was used for the assays. The precise amount was selected to provide the same amount of protein in paired normal and tumor assays. The quantity of protein used varied between 0.2 and 0.8 mg for different patients. The formation of tritiated water from ^{3}H-dUMP was shown to be linear for the 20-minute incubation period and proportional to the protein concentration of the added tissue preparations. TdR (Sigma Chemical Co, St. Louis, MO) or 5-FU was added to the tissue preparations which were then mixed with an equal volume of the "reductase mixture" of Kent and Heidelberger (4) containing: *N*-2-hydroxy-ethylpiperazine-*N*′-2-ethanesulfonic acid (pH 7.6), 100 mM; adenosine-5′-triphosphate, 6 mM; magnesium acetate, 6 mM; dithioerythritol, 8 mM; sodium fluoride, 8 mM; and ferrous ammonium sulfate, 0.02 mM. The final 5-FU and TdR concentrations were 1×10^{-5} M. These mixtures were incubated at 37°C for 5 minutes. After incubation 20 µl was mixed with 20 µl of Roberts' reagent B. The reagents, as described by Roberts, consisted of two parts, A and B. Reagent A was a stock solution containing tetrahydrofolate, 8.6×10^{-4} M; 2-mercaptoethanol, 0.075 M; Tris buffer, 0.235 M (pH 7.5); and formaldehyde, 0.43% (pH adjusted to 7.5). Fresh reagent was prepared each week and was stored frozen. Reagent B contained 250 µl of 1.0 M sodium fluoride; 500 µl of Tris buffer (pH 7.5); 500 µl of reagent A; and 220 µl of ^{3}H-dUMP of a concentration appropriate to give the desired final molar concentration. Reagent B was prepared fresh just prior to use. The final concentration of ^{3}H-dUMP (specific activity, 12.7 curies/mmol) used in the assay was 1.95×10^{-6} M. Commercial ^{3}H-dUMP was lyophilized and reconstituted before use to reduce background. No unlabeled dUMP was added. The formation of tritiated water was determined after 20 minutes' incubation at 37°C. The reaction was terminated by the addition of 0.4 ml of an equal mixture of HCl-washed charcoal (Sigma Chemical Co) and distilled water and was centrifuged at 200 × *g* for 10 minutes. One-hundred-microliter aliquots of the supernatant were counted in ACS scintillation counting fluid (Amersham-Searle, Arlington Heights, IL) in a liquid scintillation counter. Confirmation of the radioactivity in the charcoal supernatant as tritiated water was accomplished during methologic development by demonstrating that 98%–99% of these counts were removed by lyophilization or evaporation. Replicate determinations were found to vary so slightly (< 5%) that only single assays were done in the present experiments. The addition of the "ribonucleotide reductase mixture" was shown not to affect the assay of thymidylate synthetase in the absence of 5-FU.

RESULTS

TdR (1×10^{-5} M) did not affect thymidylate synthetase activity except in the case of a colonic carcinoma which grew in a patient receiving 5-FU chemotherapy (patient 5, table 1). 5-FU inhibited thymidylate synthetase activity in all but one instance. Concomitant administration of equimolar amounts of TdR and 5-FU approximately doubled the inhibition of thymidylate synthetase obtained from 5-FU alone (table 1).

DISCUSSION

5-FU's inhibition of thymidylate synthetase was enhanced by TdR, which indicates either utilization of TdR's deoxyribose for deoxyribosylation of 5-FU or modification of catabolism of 5-FU or its metabolites. TdR enhancement of 5-FU incorporation into RNA would not explain the present findings. De Verdier and Potter described deoxyribosylation between deoxynucleotides and pyrimidine bases but this did not occur between 5-FU and TdR (18). Alteration of the catabolism of 5-FU or of some 5-FU metabolites by TdR or by a TdR metabolite would appear to be a likely explanation of the enhancement of 5-FU by concomitant TdR administration.

Although administration of TdR together with 5-FU substantially increased the inhibition of thymidylate synthetase over that obtained by 5-FU alone, similar effects were found in normal and tumor tissues. 5-FU's selectivity probably is determined more on the basis of different rates of 5-FU or FdUMP catabolism, or of different rates of tumor and normal tissue recovery, than on the basis of either initial FdUMP formation or of thymidylate synthetase inhibition (19,20). TdR could protect 5-FU from catabolism by competing for nucleoside phosphorylase, or it could protect FdUMP from dephosphorylation after it is converted to TMP by thymidine kinase. In the latter case, TdR augmentation would depend upon the TdR kinase activity of any given tissue. Some human colonic carcinomas were found to dephosphorylate suf-

TABLE 1.—Effect of TdR, 5-FU, and TdR + 5-FU on human tissue extracts

Tissue	Thymidylate synthetase (pmols/mg of protein)	% inhibition‡ by—		
		1×10^{-5} M TdR	1×10^{-5} M 5-FU	TdR + 5-FU
Normal colonic tissue				
Cancer patient 2*	0	0	0	0
Patient with diverticulitis	3.46	0	45	79
Colonic cancer patient				
1	0.84	0	45	83
2	2.63	+8	+10	43
3	3.54	0	44	83
4	3.54	6	42	86
5†	32.74	23	42	88
Breast cancer patient	5.21	6	22	91
Melanoma patient	0.62	0	26	56
Lymphoma patient	4.61	0	28	83

*Normal colonic mucosal tissue obtained from colonic cancer patient 2.
†5-FU-resistant tumor.
‡+ = increased activity.

ficiently less FdUMP than did comparable normal mucosa resulting in higher and more prolonged FdUMP levels in tumors than in normal tissue (21). This differential may be enhanced by nucleotides which compete for catabolic phosphatases (22) and thereby protect FdUMP from dephosphorylation.

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Pharmacokinetics of Tallysomycin and Bleomycin in the Beagle Dog [1,2]

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SUMMARY

The pharmacokinetics of tallysomycin, a third-generation bleomycin analog, and bleomycin have been determined and compared in the beagle dog. Both compounds exhibited biphasic plasma elimination characteristics and were extensively absorbed after im injection. The elimination half-lives of tallysomycin after iv and im administration were 1.51 ± 0.41 hours and 2.40 ± 0.667 hours respectively. These values were longer than the comparable iv (1.01 ± 0.19 hours) and im (1.12 ± 0.39 hours) elimination half-lives for bleomycin. The volume of distribution in the central compartment after iv administration was 0.111 ± 0.039 liter/kg for tallysomycin and 0.125 ± 0.0723 liter/kg for bleomycin. The total apparent volumes of distribution were 0.706 ± 0.255 liter/kg and 0.388 ± 0.245 liter/kg for tallysomycin and bleomycin respectively after iv injection. These values were significantly different ($P < 0.05$). Total urinary recovery in 24 hours for tallysomycin was significantly ($P < 0.05$) less than that for bleomycin after both iv and im injections. These observed differences in pharmacokinetic behavior may, in part, account for differences in in vivo antitumor activities and toxic effects which have been reported for these drugs.

[Cancer Treat Rep 63:1821–1827, 1979]

Tallysomycin is a new glycopeptide antitumor antibiotic structurally related to bleomycin. It has been reported to be significantly more potent than bleomycin and to have a therapeutic index superior to that of bleomycin in most in vivo tumor systems in which they have been tested, including B16 melanoma, sarcoma 180, Lewis lung carcinoma, a bleomycin-sensitive P388 leukemia, and Walker 256 carcinosarcoma (1,2). Moreover, in studies in monkeys and mice employing histopathologic methods and total lung hydroxyproline determinations, tallysomycin has been found to have less pulmonary toxicity than bleomycin at equi-effective doses (Schlein A, Schurig J, Bradner WT, et al, Bristol Laboratories, unpublished data).

The mechanism of action of tallysomycin has been reported to be similar to that of bleomycin (3). Both drugs were shown to degrade isolated DNA and intracellular DNA of cells treated in vitro. Degradation of DNA by both agents was stimulated by ferrous chloride and reducing agents. However, tallysomycin was less active against isolated DNA and slightly less cytotoxic to Novikoff hepatoma ascites cells in vitro than bleomycin. Thus, at present, there is no explanation for the increased potency of tallysomycin in vivo.

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Bleomycin has been shown to be rapidly absorbed from all sites except the bladder and gastrointestinal tract in man (4). After iv bolus or im administration to humans, bleomycin is rapidly distributed and has an elimination half-life of approximately 2 hours. It is cleared by renal mechanisms, and renal dysfunction results in prolongation of the elimination half-life (4–6). At all doses and creatinine clearances studied, a two-compartment model has been shown to be appropriate to describe bleomycin pharmacokinetics. Bleomycin pharmacokinetics have not been studied in the dog.

The purpose of the present study was to compare the pharmacokinetics of bleomycin and tallysomycin in the dog. Such studies may prove to be of value in initial clinical trials and in explaining the enhanced in vivo activity of tallysomycin and the species-related differences in toxic effects.

MATERIALS AND METHODS

Animals.—Beagle dogs of either sex weighing 11–15 kg were obtained from Marshall Farms. Each dog, fasted overnight with free access to water, was placed in a sling and indwelling catheters were placed in the saphenous vein and urinary bladder to facilitate sample collection. The dogs were given iv (cephalic vein) or im (caudal thigh muscle) injections of bleomycin, 18.5 units/m^2, or tallysomycin, 3 mg/m^2. One dog was given an iv injection of a mixture of bleomycin, 18.5 units/m^2, and tallysomycin, 3 mg/m^2. The doses used were chosen because they have been shown previously to be approximately equitoxic (1).

Sample collection.—Blood samples were collected from the saphenous vein catheter at specified intervals for 8 hours following iv drug administration and 6 hours following im drug administration. Urine was collected and pooled during these periods. The catheters were then removed and the animals placed in individual metabolism cages for an additional 16–18 hours. During this time additional blood samples were obtained via external jugular vein puncture while the urine was collected from the cage catch-pan. The blood samples were collected in heparinized tubes and centrifuged under refrigeration. The plasma was drawn off and kept frozen (–20°C) until analyzed. The pooled urine samples, collected in flasks immersed in dry ice, were thawed, the volumes determined, and aliquots frozen (–20°C) until analyzed.

Drug Analysis

Bleomycin and tallysomycin were determined using the previously described radioimmunoassays (7,8). Each assay was capable of detecting as little as 2–5 ng of drug/ml of serum and could be used to detect one drug in the presence of the other since minimal (< 0.1%) cross-reactivity between the drugs occurred (8). Specimens were diluted with phosphate buffered saline (0.01 M sodium phosphate, 0.15 M NaCl, 0.1% sodium azide, pH 7.5) to be within the range of the assay. The standards used in the assay were bleomycin sulfate (Blenoxane, lot H3242) and tallysomycin A (lot 34-F-12). The results for the bleomycin radioimmunoassay are expressed in microunits/ml concentration. The unit is based upon antibacterial activity. This is used because the specific activity varies slightly (1.3–1.7 units/mg) from lot to lot. Control specimens of known bleomycin and tallysomycin concentrations were used in all assays and each assay was performed in duplicate.

Pharmacologic Calculations

Semilogarithmic plots of serum bleomycin or tallysomycin concentration versus time following iv bolus administration were biphasic, indicative of two-compartment open-model pharmacokinetics. Serum drug concentration was taken to be representative of the central compartment drug concentration (C_1) and may be described by

$$C_1 = \frac{\text{Dose}}{V_1(\alpha - \beta)}\{(k_{21} - \alpha)\,e^{-\alpha t} + (\beta - k_{21})\,e^{-\beta t}\} \quad [1]$$

$$\alpha, \beta = \frac{(k_{12} + k_{21} + k_{10}) \pm \{(k_{12} + k_{21} + k_{10})^2 - 4 \cdot k_{21} \cdot k_{10}\}^{1/2}}{2} \quad [2]$$

where V_1 is the apparent volume of the central compartment, k_{12} and k_{21} are the first-order rate constants for transfer of drug from the central to the peripheral compartment and back respectively, k_{10} is the first-order rate constant for loss of drug from the system, and t is the time after injection. For each set of data, equation [1] was fit using nonlinear least-squares regression. The parameters k_{12}, k_{21}, k_{10}, and V_1 were iterated to obtain the best fit, each data point was weighted by the factor $1/C_i^2$, and initial estimates were obtained by computerized curve stripping using the program ESTRIP. From the nonlinear least-squares estimates of the above parameters, total serum clearance (Cl_T) was calculated according to

$$Cl_T = k_{10}V_1 \quad [3]$$

and half-life was calculated according to

$$t_{1/2} = 0.693/\beta \quad [4]$$

with β calculated using equation [2] and total volume of distribution ($V_d\beta$) calculated using

$$V_d\beta = Cl_T/\beta.$$

All clearances and volumes of distribution were normalized to body weight.

Data obtained following the im administration of either drug also appeared to be consistent with a two-compartment model. Central compartment drug concentration when the drug is given by a route requiring first-order absorption is given by

$$C_1 = \frac{k_a F \text{ dose}}{V_1} \frac{(k_{21} - \alpha)\, e^{-\alpha t}}{(\beta - \alpha)(k_a - \alpha)} + \frac{(k_{21} - \beta)\, e^{-\beta t}}{(\alpha - \beta)(k_a - \beta)} + \frac{(k_{21} - k_a)\, e^{-k_a t}}{(\alpha - k_a)(\beta - k_a)} \quad [5]$$

where k_a is the apparent first-order absorption rate constant, F is the fraction of the dose absorbed, and all other parameters are as previously defined. For each set of im data, k_{12}, k_{21}, k_{10}, V_1, and k_a were iterated to obtain the best fit, each data point was weighted by the factor $1/C_i^2$, and initial estimates were obtained through the use of ESTRIP. The fraction absorbed, F, was kept constant at 1.0 since the ratios of the areas under the plasma concentration-time curve for the im treatments to those of the iv treatments were essentially unity and the same fraction of the dose was eliminated unchanged in the urine, both indicative of 100% absorption. Finally, Cl_T, half-life, and $V_d\beta$ were calculated as above (9–12).

RESULTS

Iv Administration

Both bleomycin and tallysomycin exhibited biphasic drug elimination characteristics following iv injection (fig 1). Since computer analysis indicated that a two-compartment model best fit the data obtained from each dog, this model was selected for pharmacokinetic analyses. The pharmacokinetic parameters were determined for each of the five animals and the mean values are displayed for tallysomycin and bleomycin in table 1. The mean ± standard deviation terminal elimination half-life for tallysomycin (1.51 ± 0.41 hours) was not significantly ($0.1 < P < 0.2$) longer than that for bleomycin (1.01 ± 0.19 hours). The mean volume of distribution of the central compartment (V_1) for tallysomycin (0.111 ± 0.0393 liter/kg) was not significantly different from that for bleomycin (0.125 ± 0.0723 liter/kg). However, tallysomycin occupied a greater apparent volume of distribution in the peripheral tissues since the overall volume of distribution ($V_d\beta$) for tallysomycin (0.706 ± 0.255 liter/kg) was greater ($P < 0.10$) than that for bleomycin (0.388 ± 0.245 liter/kg).

Im Administration

Peak serum concentrations of bleomycin and tallysomycin were observed 30–60 minutes following im administration (fig 2). The initial distribution phase after im administration of the two agents was obscured by the absorption phase. The mean elimination half-life of tallysomycin (2.40 ± 0.68 hours) was significantly longer ($P < 0.05$) than the mean bleomycin elimination half-life (1.12 ± 0.39 hours) after im administration (table 2). The volume of distribution ($V_d\beta$) of tallysomycin (0.968 ± 0.413 liter/kg) was larger than that observed for bleomycin (0.293 ± 0.113) liter/kg), and both values agreed with observations after iv administration.

The im bioavailability of bleomycin and tallysomycin was determined by comparing the area under the plasma concentration-time curves and the urinary recovery data obtained after iv and im doses in the same dogs. These data suggest that either drug administered im was essentially 100% bioavailable (tables 1 and 2).

Pharmacokinetics of Bleomycin and Tallysomycin Administered Simultaneously Iv to a Single Dog

To compare the pharmacokinetics of the drugs in a system without individual animal variability, the two drugs were mixed immediately prior to iv administration to one dog and the serum and urine concentrations of each were determined with the respective assays. In this dog the elimination half-life was 2.0 hours for tallysomycin and 1.3 hours for bleomycin.

Urinary Recovery

The 24-hour urinary recovery of bleomycin after iv or im administration was 83.4% ± 12.8% or 83.0% ± 28.7% respectively. A significantly ($P < 0.01$) smaller fraction of tallysomycin was recovered after iv (19.5% ± 4.5%) and im (23.8% ± 3.1%) injections. For both drugs, > 90% of the total drug recovered in the urine in 24 hours was recovered in the first 6–8 hours after administration by each route.

The renal clearance (Q) was determined using the equation $Q = X_u^0/AUC$, where X_u^0 was the amount of drug excreted in the urine for 24 hours and AUC was the area under the serum concentration-time curve. Renal clearance for bleomycin (3.09 ± 1.2 ml/minute/kg) was significantly ($P < 0.02$) greater than for tallysomycin (1.29 ± 0.31 ml/minute/kg) following iv injection. Renal clearance following im injection was also greater for bleomycin (2.78 ± 1.2 ml/minute/kg) than for tallysomycin (1.4 ± 0.25 ml/minute/kg), although the degree of significance was less ($0.05 < P < 0.1$).

DISCUSSION

The present study has demonstrated that the pharmacokinetic behavior of tallysomycin is similar to that of bleomycin in the dog and in man (4–6,13). However, several significant differences in the drugs' pharmacokinetics were also observed, and these may, in part, explain the

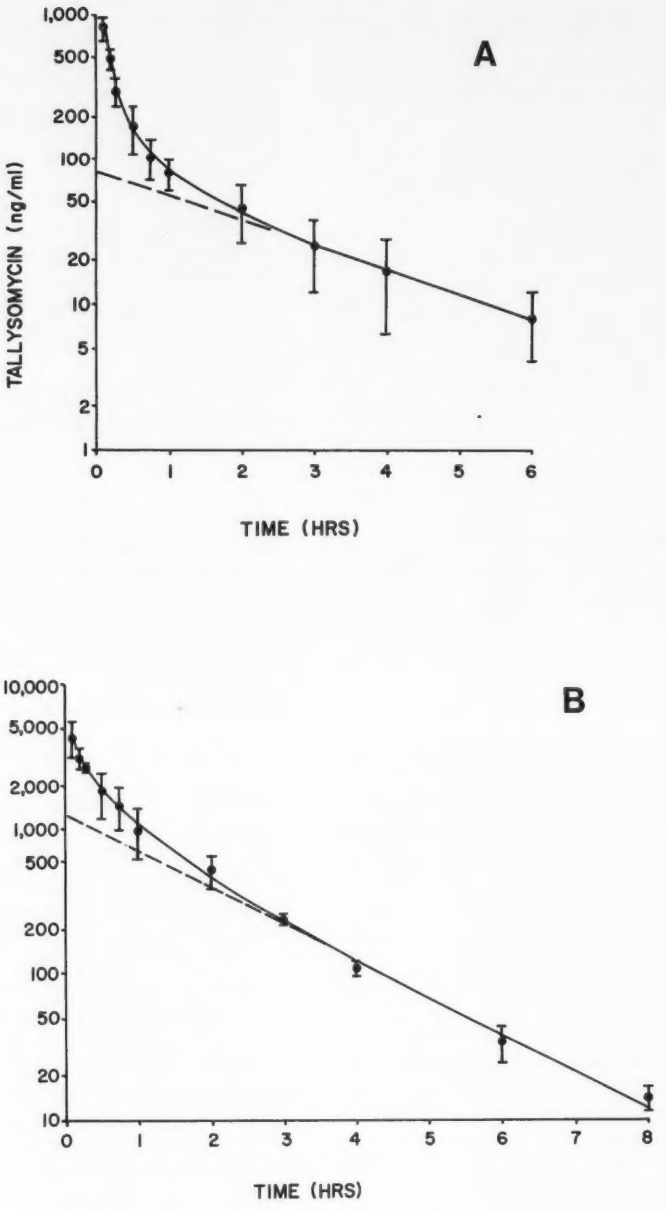

FIGURE 1.—Plasma drug levels following iv administration. Samples were collected at the indicated times and the mean values for 5 dogs, plus standard deviations, are indicated. A. Tallysomycin plasma concentrations in dogs given 3 mg/m^2 iv. B. Bleomycin plasma concentrations in dogs given 18.5 units/m^2 iv.

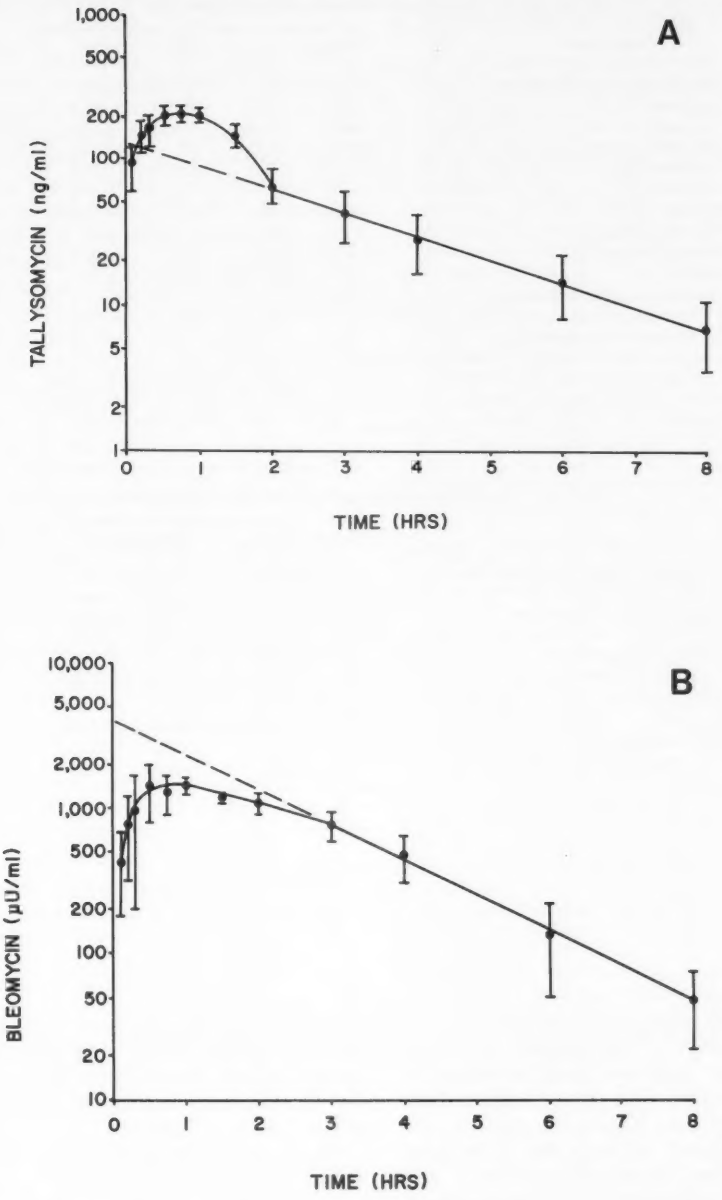

FIGURE 2.—Plasma drug levels following im administration. Samples were collected at the indicated times and the mean values for 4 dogs, plus standard deviations, are indicated. A. Tallysomycin plasma concentrations in dogs given 3 mg/m^2 im. B. Bleomycin plasma concentrations in dogs given 18.5 units/m^2 im.

TABLE 1.—Iv injection of bleomycin and tallysomycin

Parameter*	Bleomycin†	Tallysomycin†
Elimination half-life (hrs)	1.01 ± 0.194	1.51 ± 0.407
k_{12} (min^{-1})	0.107 ± 0.124	0.0477 ± 0.0215
k_{21} (min^{-1})	0.0403 ± 0.0266	0.078 ± 0.0779
k_{10} (min^{-1})	0.0373 ± 0.0181	0.0515 ± 0.0186
V_1 (liters/kg)	0.125 ± 0.0723	0.111 ± 0.0393
α (min^{-1})	0.174 ± 0.166	0.109 ± 0.0344
β (min^{-1})	0.012 ± 0.0016	0.00806 ± 0.00193
Cl_T (ml/min/kg)	3.71 ± 1.56	5.38 ± 1.37
$V_d\beta$ (liters/kg)	0.388 ± 0.245	0.706 ± 0.255
AUC‡	3956 ± 1384	428.4 ± 87.37
Renal clearance (ml/min/kg)	3.09 ± 1.2	1.29 ± 0.31

*See text for definitions.

†Nos. are the means ± standard deviation for 5 dogs.

‡Area under the curve (AUC) is calculated from the fitted parameters, and units are expressed as microunits-hr/ml for bleomycin and ng-hr/ml for tallysomycin.

TABLE 2.—Im injection of bleomycin and tallysomycin

Parameter*	Bleomycin†	Tallysomycin†
Elimination half-life (hrs)	1.12 ± 0.389	2.40 ± 0.677
k_{12} (min^{-1})	0.0195 ± 0.013	0.0168 ± 0.00709
k_{21} (min^{-1})	0.0405 ± 0.166	0.00971 ± 0.00411
k_{10} (min^{-1})	0.0222 ± 0.0114	0.0285 ± 0.0131
V_1 (liters/kg)	0.176 ± 0.101	0.178 ± 0.0562
k_a (min^{-1})	0.0207 ± 0.0115	0.0270 ± 0.00992
OC (min^{-1})	0.0203 ± 0.0212	0.0499 ± 0.0163
β (min^{-1})	0.0118 ± 0.00574	0.00510 ± 0.00140
Cl_T (ml/min/kg)	3.04 ± 0.357	4.54 ± 0.715
$V_d\beta$ (liters/kg)	0.293 ± 0.113	0.968 ± 0.413
AUC‡	4263 ± 687.4	469.1 ± 63.8
Renal clearance (ml/min/kg)	2.78 ± 1.19	1.4 ± 0.25

*See text for definitions.

†Nos. are the means ± standard deviation for 4 dogs.

‡Area under the curve (AUC) is calculated from the fitted parameters, and units are expressed as microunits-hr/ml for bleomycin and ng-hr/ml for tallysomycin.

previously reported differences in in vivo antitumor activities and toxic effects (1,2). Both bleomycin and tallysomycin exhibited biphasic drug elimination characteristics following iv or im administration. Both drugs were extensively absorbed after im injection. Although both drugs exhibited relatively short elimination half-lives, the elimination half-life of tallysomycin was longer than that of bleomycin after iv and im injections. Furthermore, the urinary recovery of bleomycin was > 80% of the administered dose irrespective of route and that of tallysomycin was approximately 20% irrespective of route. The fact that the iv elimination half-life of tallysomycin was not statistically significant at the $P < 0.05$ level is probably due to a relatively larger standard error in the tallysomycin-treated dog samples. However, the im elimination data, the data from the dog that received both drugs simultaneously iv, and unpublished data in monkeys showing a statistically significantly longer elimination half-life for iv administered tallysomycin compared to bleomycin confirm the data suggesting a longer iv half-life for tallysomycin.

The increased elimination half-life and reduced 24-hour urinary recovery of tallysomycin may have resulted from enhanced uptake of tallysomycin by peripheral tissues. Although the volume of distribution of the central compartment of tallysomycin was not different from that of bleomycin, the total apparent volume of distribution ($V_d\beta$) of tallysomycin was significantly larger. Thus, the volume of distribution of the peripheral compartment of tallysomycin must have been larger than that of bleomycin. This difference may have resulted from the avidity with which these drugs bind to their principal intracel-

lular receptor DNA (3,14,15). Since tallysomycin has been shown to have a 20-fold greater affinity for salmon sperm DNA than bleomycin A_2 (3), increased DNA binding could have resulted in enhanced retention of tallysomycin in the peripheral compartment. No serum protein binding for either drug was detected in the serum.

Another possible explanation for the lower percentage of tallysomycin recovered in the urine is that a greater percentage of tallysomycin than bleomycin was metabolized to immunochemically nonreactive metabolites. Although metabolic products of bleomycin have been reported (16), the primary method of elimination is renal excretion, and the major metabolite has been reported to cross-react in the radioimmunoassay for bleomycin (17). The longer retention of tallysomycin in the peripheral tissues may have allowed more extensive metabolism of tallysomycin, and metabolites may not cross-react with the tallysomycin radioimmunoassay antiserum.

Whether the reduced urinary recovery of tallysomycin was caused by increased metabolism or irreversible tissue uptake, a nonrenal mechanism of elimination appears more important for tallysomycin than for bleomycin. This was reflected in the total clearance and renal clearance of both drugs. Bleomycin was primarily removed from the body by renal clearance following im and iv administration (> 80%). However, tallysomycin renal clearance accounted for < 30% of the total clearance irrespective of administration route. Nonrenal mechanisms could have involved more extensive metabolism or irreversible tissue binding.

Future studies designed to compare the pharmacokinetics of tallysomycin and bleomycin in man should determine whether these results found in the dog model are also valid in patients. However, based upon the present findings, tallysomycin appears more widely distributed in the peripheral tissues and less dependent upon renal elimination pathways when compared to bleomycin.

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A Radioimmunoassay for Tallysomycin[1,2,3]

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SUMMARY

Antibodies were raised against a new antineoplastic agent, tallysomycin, for use in a radioimmunoassay. The assay was sensitive to 2.5 ng of tallysomycin, had a coefficient of variation of 10.5% at 50 ng/ml, and was highly specific for tallysomycin A. The antibody exhibited reduced cross-reactivity with closely related tallysomycin analogs and little or no immunoreactivity with other antitumor antibiotics including bleomycin and phleomycin. Increased immunoreactivity occurred following copper chelation of tallysomycin B as compared with the non-chelated forms, thus indicating possible conformational alterations upon metal binding.

[Cancer Treat Rep 63:1829–1832, 1979]

The first of the third-generation bleomycin analogs to be characterized and developed is tallysomycin, a new water-soluble basic antibiotic complex extracted from the fermentation broth of a *Streptomyces*-like organism isolated from an Indian soil sample (1). Tallysomycin consists of two major components, A and B. Their structures have been identified (fig 1) and were found to be similar to the bleomycin complex. Tallysomycin was significantly more potent than bleomycin as an antibacterial and antifungal agent (2). The drug was also a more potent antitumor agent in experimental animal tumor systems including P388 lymphocytic leukemia, sarcoma 180, Lewis lung carcinoma, Walker 256 carcinosarcoma, and B16 melanoma (1).

Tallysomycin is currently undergoing preclinical toxicologic evaluation in preparation for clinical trials. Consequently, it is essential that a sensitive method for pharmacokinetic determinations be developed. Microbiologic assays, generally used for determinations of antibiotic levels, are useful in detecting biologic activity but are limited in their sensitivity and specificity. A radioimmunoassay was developed for bleomycin (3) that was sensitive, specific, rapid, and useful for monitoring bleomycin in plasma and urine (4). This report describes the development of a radioimmunoassay for tallysomycin that is suitable for monitoring drug levels in biologic fluids and should facilitate pharmacokinetic investigations of drug distribution. The assay specificity was investigated with several different chemotherapeutic agents and analogs of tallysomycin.

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[2] Presented in part at the 62nd annual meeting of the Federation of American Society for Experimental Biology, Atlantic City, NJ, Apr 1978 (*see* ref 11).

[3] Supported by a grant from Bristol Laboratories, Syracuse, NY.

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MATERIALS AND METHODS

Preparation of a Tallysomycin-Bovine Serum Albumin Conjugate

Tallysomycin, A > 95% purified (generously supplied by Bristol Laboratories, Syracuse, NY), was conjugated to bovine serum albumin (BSA) by a method previously described for bleomycin (3). The procedure included use of 1-ethyl-3-(dimethylaminopropyl)-carbodiimide for conjugation followed by chromatographic purification on Sephadex G25.

Immunization

The purified tallysomycin-BSA conjugate was used for antisera production in three New Zealand white rabbits

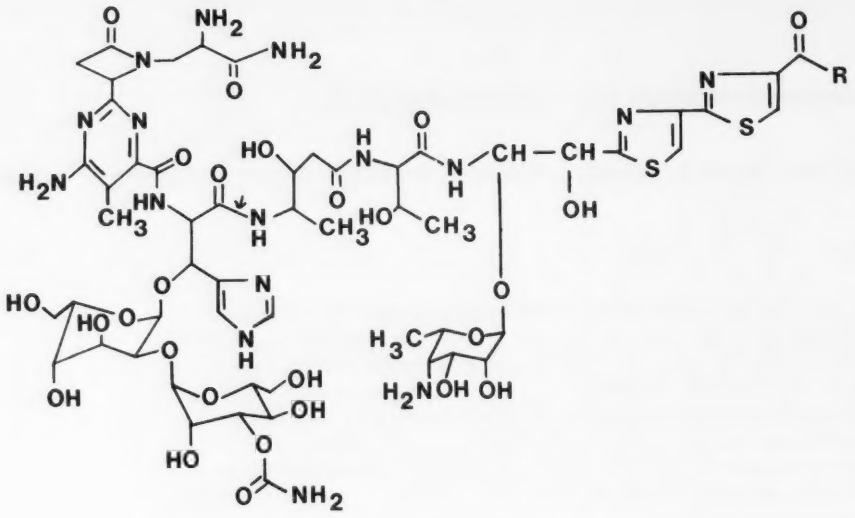

FIGURE 1.—Structural formula of the tallysomycins. Tallysomycin A: R = $NH(CH_2)_3CH(NH_2)CH_2\overset{\overset{O}{\|}}{C}NH(CH_2)_3NH(CH_2)_4NH_2$. Tallysomycin B: R = $NH(CH_2)_3NH(CH_2)_4NH_2$. Peptide Wa and Wb are hydrolysis products of tallysomycin A and B respectively. The site of hydrolysis is indicated by an arrow. Tallysomycin E_{1a}: R = $NH(CH_2)_3CH(NH_2)CH_2\overset{\overset{O}{\|}}{C}NH(CH_2)_3NH_2$. Tallysomycin S_{2b}: R = $NH(CH_2)_3S^+(CH_3)_2$.

by the procedure of Hurn and Landon (5). Each rabbit received a total of 2.0 mg of conjugate mixed in an aqueous emulsion of Freund's complete adjuvant (Difco Laboratories, Detroit, MI) and injected im (1 ml) into all limbs. Monthly booster inoculations of 1.0 mg of conjugate were injected into either the hind or fore limbs.

Iodination

Tallysomycin was iodinated as previously described for bleomycin (3). The iodinated product was purified by cation exchange chromatography. The following ingredients were mixed and incubated at room temperature for 3 minutes: 10 µl of $Na^{125}I$ (100 millicuries/ml), 10 µl of chloramine T (5-mg/ml solution in borate buffer, pH 9.0), and 10 µl of tallysomycin A (1.5 mg/ml in 0.1 M borate buffer, pH 9.0). Next, 10 µl of sodium metabisulfite (12-mg/ml solution in 0.1 M borate buffer, pH 9.0) was added followed by 10 µl of potassium iodide (20-mg/ml solution in 0.1 M borate buffer, pH 9.0).

The iodinated tallysomycin was separated from the reaction ingredients by column chromatography on CM Sephadex G25 (30 × 1.2 cm) using a gradient of ammonium formate (pH 6.4) from 0.1 to 1.0 M at a flow rate of 1 ml/minute followed by elution of the labeled tallysomycin with ammonium formate (1.0 M) at a reduced flow rate of 0.25 ml/minute. One-milliliter fractions were collected and fractions 80–90 contained the iodinated tallysomycin. The dissociation of iodine 125 from the labeled tallysomycin resulted in approximately 5% background radioactivity that was not precipitable by charcoal or bound by antibody. This figure increased to 15% after approximately 6 weeks of incubation.

Radioimmunoassay Procedure

A competitive protein-binding assay between ^{125}I-labeled tallysomycin and unlabeled tallysomycin for antibody binding sites was used to quantitate unknown concentrations of tallysomycin in various fluids. Antibody-bound drug was separated from unbound drug following dextran-coated charcoal adsorption of free tallysomycin.

The radioimmunoassay of tallysomycin was performed using the following protocol in the order given: 200 µl of 0.1% gelatin dissolved in phosphate buffered saline (PBS),[7] 100 µl of sample or appropriate standards, 100 µl of ^{125}I-labeled tallysomycin diluted to approximately 30,000 cpm in PBS, and 100 µl of antiserum diluted

[7] 0.01 M sodium phosphate, pH 7.5; 0.15 M NaCl; and 0.1% sodium azide.

(1:5000) to bind approximately 50% of the iodinated drug in 0.1% gelatin dissolved in PBS were thoroughly mixed and incubated (30 minutes at 37°C; 15 minutes at 4°C). The standard solutions of tallysomycin in PBS were prepared from a stock solution of tallysomycin A (100 μg/ml) in PBS to cover a range of 10–300 ng/ml (1.0–30.0 ng/tube). Following incubation, 200 μl of dextran-coated charcoal (2 g of dextran T-70 and 2 g of activated charcoal in 100 ml of PBS) plus 400 μl of PBS at 4°C were added and the mixture was centrifuged (2000 × *g*, 10 minutes, 4°C). Supernatants were decanted and the radioactivity was measured in an automatic gamma spectrometer (Nuclear Chicago Corp, Des Plaines, IL).

RESULTS

The immunogen used to produce tallysomycin antisera contained a molar ratio of tallysomycin:BSA estimated spectrophotometrically to be 28:1. After three booster injections, the antiserum titer, determined by the highest antiserum dilution which bound 50% of ^{125}I-labeled tallysomycin, was 1:50,000. The affinity of antiserum for tallysomycin was determined by Scatchard analysis (3,6) and was found to be 8.5×10^8 liters/mol^{-1}. The specific activity of the iodinated tallysomycin was approximately 5 microcuries/mg, resulting in an incorporation of approximately 3×10^{-6} atoms of iodine 125 per molecule of tallysomycin.

Standard curves for the tallysomycin radioimmunoassay were linear on a logit-log plot (fig 2). The sensitivity of the assay, defined as the lowest amount of tallysomycin that could be significantly ($P < 0.05$) detected, was 2.5 ng (± 1.44). The precision of the assay at 50 ng was determined for ten replicate samples, with a resulting coefficient of variation of 10.5%. The specificity of the radioimmunoassay was investigated in cross-reactivity experiments using a variety of antineoplastic agents as well as tallysomycin analogs (table 1). Minimal cross-reactivity occurred with other antineoplastic drugs. Even bleomycin A_2, differing from tallysomycin by the absence of a talose sugar and having a different terminal amide, did not cross-react. The importance of the terminal group in immunoreactivity was demonstrated by a threefold reduction in cross-reactivity for tallysomycin B (with copper) and the negligible cross-reactivity by tallysomycin E_{1a} and S_{2b}. These compounds differ from tallysomycin A primarily by variation in their terminal group structures. The tallysomycin A hydrolysis product, peptide Wa, containing the same terminal group plus four adjacent peptides from the parent compound, cross-reacted with 62% efficiency, thus also indicating the immunodominance of the terminal group structure and adjacent peptides.

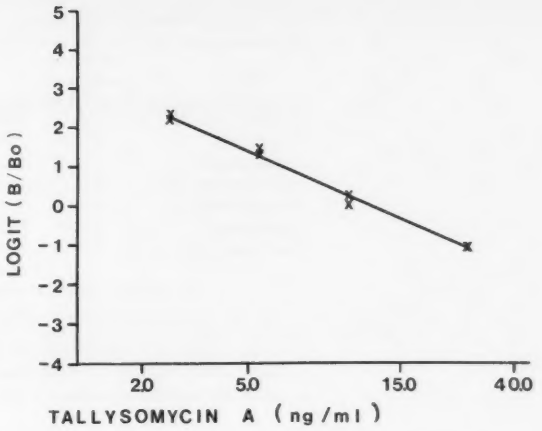

FIGURE 2.—Standard curve for tallysomycin A on a logit-log plot. Logit B/Bo = logit of the ratio of antibody-bound radioactivity to radioactivity bound at 0 concentration of unlabeled tallysomycin where logit $B/Bo = \ln\left(\frac{B/Bo}{100 - B/Bo}\right)$.

DISCUSSION

A radioimmunoassay for bleomycin allowed detection of the drug at concentrations lower than the previously employed microbiologic assays (7). This assay has facilitated detailed analysis of the pharmacokinetics of bleomycin when administered to patients by various routes (4,8). The tallysomycin radioimmunoassay will allow investigation of preclinical and clinical tallysomycin pharmacokinetics. It is now possible to perform pharmacokinetic and tissue distribution studies in animals given the two drugs simultaneously, thus eliminating inter-animal variations, because the bleomycin and tallysomycin radioimmunoassays do not cross-react. Studies on the cross-reactivity of the antisera to other antitumor agents demonstrate that the radioimmunoassay for tallysomycin is highly specific and that the assay will be useful in patients receiving a variety of drugs.

The cross-reactivity of the tallysomycin antisera correlates well with the previous reports from this laboratory on the specificity of the bleomycin antisera (9). This assay and the bleomycin radioimmunoassay are both sensitive to alterations in the terminal group structure of the molecule. However, changes in the tallysomycinic acid nucleus are also important since peptide Wa and Wb, containing the terminal group structures of tallysomycin A and B respectively, exhibited reduced cross-reactivity.

Conformational changes in tallysomycin B following copper complexation may be indicated by the increased immunoreactivity of the copper-chelated form (table 1). However, copper-chelated bleomycin A_2 had the same

TABLE 1.—Cross-reactivity* of tallysomycin antisera

Drug	% cross-reactivity	Drug	% cross-reactivity
Tallysomycin A	100	Bleomycin	< 0.1
Vincristine	< 0.1	Desamido bleomycin	< 0.1
Peptichemio	< 0.1	Phleomycin	< 0.1
Neocarzinostatin	< 0.1	Tallysomycin A with copper	100
Methotrexate	< 0.1	Peptide Wa	62
Adriamycin	< 0.1	Tallysomycin B with copper	33
Carminomycin	< 0.1	Tallysomycin B	5.0
		Tallysomycin E_{1a} with copper	5.0
		Tallysomycin S_{2b} with copper	< 1.0
		Peptide Wb	5.0

*Cross-reactivity was defined as the molar concentration of drug required to produce 50% inhibition of ^{125}I-labeled tallysomycin bound by antiserum relative to unlabeled tallysomycin A.

immunoreactivity as the copper-free form in the bleomycin immunoassay. Similar results were obtained for copper-chelated tallysomycin A in the tallysomycin assay.

Since tallysomycin B differs from tallysomycin A by a β-lysine adjacent to the terminal amine structure, the sixfold increase in the cross-reactivity of tallysomycin B copper chelation reflects the sensitivity of the assay to changes in that portion of the molecule associated with binding of copper. Preliminary studies have shown that 1 mol of tallysomycin B binds to 2 mols of cuprous ion. The first molecule of Cu^{++} binds at the site identified in the bleomycin molecule (10); the second may bind to other sites of the molecule. Thus, significant conformational changes may be induced by the binding of Cu^{++}.

The biologically less active bleomycin metabolite, desamido bleomycin, has been shown to cross-react with the bleomycin antibody. At present, no metabolites of tallysomycin have been identified, but similar cross-reactivity in the tallysomycin assay can be predicted since the partial degradation product, peptide Wa, containing the immunodominant terminal amine, cross-reacts in this assay. Therefore, to determine whether the drug's immunoreactivity correlates with biologic activity, it will be necessary to compare the results obtained using this radioimmunoassay with those obtained using a microbiologic assay in biologic fluids following drug administration.

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ADDENDUM

The tallysomycin structure is now thought to contain a ring opened β-lactam system rather than a β-lactam moiety.

Ocular Assessment of Patients Treated With Tamoxifen [1]

Marilyn Beck* and Paul V. Mills [2]

SUMMARY

Tamoxifen is a specific estrogen antagonist used in the treatment of breast cancer. In a previous study, corneal and retinal changes were reported in four patients receiving high-dose tamoxifen therapy for > 1 year. Nineteen patients treated with tamoxifen for periods of 3 months to 4 years at normal dose levels have been studied and no ocular changes attributable to the drug were observed.

[Cancer Treat Rep 63:1833–1834, 1979]

Tamoxifen, a specific estrogen antagonist, has been used in the palliative treatment of breast cancer for the past 8 years. The recommended oral dose is 10 mg twice daily, increasing to 20 mg twice daily if no response is obtained within 1 month (1,2). No serious side effects had been reported (3–6) until Kaiser-Kupfer and Lippman (7) described ocular abnormalities in four patients receiving high doses of tamoxifen (120–160 mg twice daily) for > 1 year. All four patients were found to have a retinopathy consisting of small white refractile opacities in the macular and paramacular areas, associated with decreased visual acuity in three patients. Three of the same patients were also found to have whorl-like superficial corneal opacities.

The present report concerns the assessment of patients receiving lower doses of tamoxifen for evidence of ocular toxicity.

PATIENTS AND METHODS

Nineteen female patients between 54 and 84 years of age were studied. All patients had histologically proven breast carcinoma with signs of recurrence after the primary therapy and all had been treated with tamoxifen in doses not exceeding 20 mg twice daily for periods of between 3 months and 4 years.

All patients were interviewed and examined independently by both authors. The current ocular symptoms and past ocular and medical histories were obtained. Details of the dose and duration of tamoxifen therapy, other medication taken with tamoxifen, and any previous long-term medication were noted. The corrected visual acuity in each eye for distant and near vision was recorded and a full ocular examination was undertaken. Tests of macular function included the Hardy-Rand-Rittler color vision test and the red on black Amsler chart. All patients were examined with a slit-lamp biomicroscope, and the quality of the tear film and any abnormalities in the conjunctiva, cornea, or lens were noted. The intra-ocular pressure was measured by applanation tonometry. The fundi were examined following dilatation of the pupils, using both the direct and binocular indirect ophthalmoscope, together with a Goldmann three-mirror contact lens if indicated.

RESULTS

The results of this investigation are detailed in table 1. None of the patients studied showed either the whorl-like superficial corneal opacities or a retinopathy of the type described following exposure to high-dose tamoxifen. In all of the patients with an impaired visual acuity a lesion was present, unrelated to the tamoxifen therapy, consistent with the visual deficit. No new side effects were found in any patient.

DISCUSSION

Ocular toxicity in the form of a keratopathy associated with a retinopathy has been reported recently by Kaiser-Kupfer and Lippman (7) in four patients receiving very

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TABLE 1.—Summary of results

Patient No./Age	Duration of treatment* (mos)	Visual acuity		Posterior pole/ macular appearance†		Other ocular pathology	
		Right eye	Left eye	Right eye	Left eye	Right eye	Left eye
1/68	7	20/20	20/15	a	—	—	—
2/73	48	20/30	20/40	a,b	a,b	f	g
3/57	14	20/20	20/20	—	b	—	—
4/74	26	20/30	20/40	—	—	f	f
5/77	21	20/30	20/30	—	—	h	h
6/54	22	20/20	20/30	b	—	—	g
7/62	9	20/20	20/20	a	—	—	—
8/84	17	20/15	20/20	a	a,b	—	—
9/57	12	20/20	20/15	—	—	—	—
10/65	15	20/20	20/20	b	—	—	—
11/79	13	CF‡	3/100	d	d	—	—
12/73	20	20/20	20/20	b	—	—	—
13/81	12	20/25	20/30	b	b	f	f
14/63	17	20/15	20/15	b	b	—	—
15/71	3	CF‡	20/30	b,e	—	f	f
16/80	3	3/200	10/200	c,d	b	—	—
17/57	12	20/15	20/15	b	b	—	—
18/54	13	20/15	20/20	—	—	—	—
19/59	13	20/20	20/20	—	b	—	—

*Dose of tamoxifen = 20 mg twice daily except for patient 14 who received 10 mg twice daily.

†a = drusen, b = pigment changes, c = disciform degeneration, d = macular hole, e = myopic degeneration, f = lens opacity, g = amblyopia, h = keratitis sicca.

‡CF = counting fingers.

high doses of tamoxifen. They state that the adverse effects of tamoxifen appear to be related to high drug levels and recommend that the eyes of patients receiving this medication be examined periodically. It was not known, however, whether these ocular changes would occur with lower doses of this drug.

In the present study, the average duration of treatment was 15 months and the initial dose of tamoxifen was 10 mg twice daily increasing in all but one patient to 20 mg twice daily. At this dose level no patient showed evidence of ocular toxicity, although 11 of the 19 women obtained complete clinical regression of the metastatic carcinoma of the breast.

Because high doses of this drug have been incriminated in the development of retinal and corneal changes, the usefulness of tamoxifen given in such high doses will need to be demonstrated.

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Effect of Mithramycin on Widespread Painful Bone Metastases in Cancer of the Breast [1]

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SUMMARY

Fifteen patients with widespread painful osseous metastases from breast cancer unresponsive to other systemic therapy were treated with mithramycin at dose levels usually used for treating Paget's disease. Ten patients had relief of pain, which was marked and rapid in onset in seven. Mobility was greatly improved in four patients. Healing of bone lesions did not occur and new lesions developed while treatment was being given. Clinical response was associated with a decrease in plasma alkaline phosphatase. Toxicity was mild and consisted of nausea in most patients and a slight decrease in platelet count in one patient. Mithramycin is a useful agent for palliation of painful bone metastases and should be considered for further trials of combination chemotherapy for advanced breast cancer with bone metastases.

[Cancer Treat Rep 63:1835–1838, 1979]

Breast cancer may spread to the bone, causing severe pain and inhibiting mobility. Mithramycin has been shown to inhibit breast cancer-induced osteolysis (1) and is known to relieve severe bone pain in myelomatosis (2), Paget's disease (3), and parathyroid carcinoma (4). In contrast to the high doses required for its use as an antitumor agent, which are associated with severe toxicity, the doses required to reduce pain in Paget's disease are low and toxicity is slight. For these reasons we have used mithramycin as a single agent in a group of 15 patients with advanced breast cancer and widespread bone metastases in whom other systemic treatments had failed and who had severe generalized bone pain.

PATIENTS AND METHODS

All patients were known to have breast cancer proven at mastectomy or by biopsy. All had severe widespread bone pain due to secondary deposits demonstrated on roentgenograms and radioisotope bone scans. Patients with other known skeletal diseases were excluded from the study, and none of the patients had significant impairment of the renal, endocrine, or cardiovascular system. Previous treatments were various and included hormone therapy, cytotoxic chemotherapy, steroids, and radiotherapy. In all patients except one these other treatments had lost their effectiveness in controlling the disease. Fifteen patients were treated and their ages ranged from 48 to 78 years. Ten patients were postmenopausal when the original diagnosis was made and five were premenopausal.

Blood was taken before and after each treatment with mithramycin and measurements of full blood cell count, platelets, plasma urea and electrolytes, plasma creatinine, calcium, phosphate, and alkaline phosphatase were made on each occasion. In 12 patients, total hydroxyproline was measured in 24-hour urine samples before and after using the method of Coward et al (5). In six patients, total urinary calcium was measured in 24-hour urine collections before and after treatment. In all patients skeletal roentgenograms and radioisotope bone scans were done before treatment was started and were repeated at approximately 3-month intervals. The patients were admitted to the hospital and received mithramycin iv at a dose of 15 μg/kg/day diluted in 500 ml of 5% dextrose solution given over a period of 4 hours daily on each of

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3 successive days. They were then discharged and readmitted 3 weeks later, at which time the cycle was repeated. Patients received between one and six cycles at 3-week intervals depending on their response, general condition, and the development of side effects. Four patients then went on to receive 15 μg/kg given over 1 hour once weekly on an outpatient basis for periods ranging from 4 to 15 weeks. Before each treatment, patients were questioned by the same doctor regarding site, frequency, and severity of pain and a questionnaire was completed assessing degree of mobility and consumption of analgesics.

RESULTS

Effect on Pain and Mobility

Fifteen patients were treated and 14 had more than one course of treatment. Ten patients experienced relief of pain. Seven had considerable and immediate relief with improvement in mobility, while another three had partial relief of pain. Analgesic consumption decreased in ten patients. The detailed results are shown in tables 1 and 2.

TABLE 1.—Relief of pain with mithramycin

Pain relief	Incidence*
Complete relief of pain	4/15
Partial relief of pain with increased mobility	3/15
Partial relief of pain alone	3/15
No improvement	2/15†
Pain worse	3/15

*No. of patients with indicated effect/No. treated.
†In both of these patients mithramycin was stopped after only 2 cycles because of unacceptable nausea.

TABLE 2.—Improvement in mobility with mithramycin

Degree of disability	No. of patients with indicated degree	
	Before treatment	After treatment
Immobile	5	2
Able to do simple functions (able to stand, walk, and wash herself, but unable to walk very far and still housebound)	3	2
Able to walk short distances (unable to shop or carry weights)	5	6
Normal mobility	2	5

Adverse Effects

Three patients felt that their pain had worsened while receiving mithramycin and they attributed this to the drug. Nausea was very common and in two patients led to the treatment being discontinued. In general, the nausea was not a problem, particularly in patients receiving the hourly weekly infusion on an outpatient basis. On the 3-day regimen, hematologic toxicity was confined to a slight decrease in platelet count in two patients and resulted in dose reduction in one patient. The wbc counts remained within the normal range and no hemorrhagic complications were observed (table 3). On the daily regimen (used in four patients) the platelet count fell to $< 100{,}000/mm^3$ but was $> 90{,}000/mm^3$ in one patient on two occasions among a total of 40 treatments.

LABORATORY INVESTIGATIONS

Roentgenograms and bone scans were repeated at approximately 3-month intervals following therapy, but there was no evidence of bone healing and an increase in size and quantity of metastases both radiologically and on scanning occurred in several patients.

Alkaline phosphatase levels were elevated before treatment in ten patients. Levels fell in eight patients and seven of these gained considerable relief of pain (tables 4 and 5). Levels rose in two patients and neither of these obtained any relief of pain. In five patients, levels were normal before and after therapy, and of these five, only two had relief of pain. Of the eight patients in whom

TABLE 3.—Hematologic effects of mithramycin*

	Start of treatment	End of treatment
Mean hemoglobin (g/dl)	12.1	11.8
Mean wbc count (cells × 10^9/liter)	6.47	6.1
Mean platelet count (cells × 10^9/liter)	222.5	182

*Results refer to the 3-day regimen. Total No. of cycles = 42.

TABLE 4.—Pain relief with mithramycin related to effect on alkaline phosphatase

Effect on alkaline phosphatase	No. of patients	No. with relief of pain
Alkaline phosphatase increased after mithramycin	2	0
Alkaline phosphatase decreased after mithramycin	8	7
Alkaline phosphatase unchanged after mithramycin	5	2

TABLE 5.—Effect of mithramycin on plasma calcium and alkaline phosphatase

	Values—*		
	Before treatment	After 1st treatment	After last treatment
Calcium (normal = 2.3–2.7 mmols/liter)	2.60 ± 0.09	2.39 ± 0.05	2.37 ± 0.07
Alkaline phosphatase (normal = 22–105 IU/liter)	283.6 ± 86.2	245.6 ± 56.2	228.1 ± 51.1

*Values are mean mmols/liter ± SE.

alkaline phosphatase levels fell, four had elevated alanine transaminase levels and two had elevated gamma glutamyl transpeptidase levels. Neither of these more liver-specific enzymes fell after mithramycin therapy. It is therefore probable that the decrease in alkaline phosphatase seen in these eight patients reflected a change in bone biochemistry.

Serum calcium levels fell in all patients after each mithramycin infusion (table 5). This effect on serum calcium was found to be temporary and not cumulative. Symptomatic hypocalcemia occurred on only one occasion but symptoms disappeared with no therapy after a few hours' rest.

Urinary excretion of hydroxyproline was measured on 24-hour urine collections in 12 patients, but only two of these patients had elevations before treatment and neither had a decrease following treatment. The other ten patients had normal hydroxyproline excretion before and after treatment. This result is surprising, since urinary hydroxyproline excretion is reported as being increased in many patients with bone metastases (6). No change was discovered in urinary calcium excretion measured in 24-hour urine collections before and after treatment in six patients. Renal function was normal in all patients.

DISCUSSION

Osteolytic metastases are common in breast cancer and are an important cause of morbidity. Treatment with radiotherapy is effective in relieving local pain but is impractical in patients with widespread painful deposits. Relief of pain and even healing of lytic lesions may sometimes be achieved by hormone manipulation.

Bone destruction in breast cancer appears to be caused by at least two separate mechanisms. Cultured breast cancer cells have been shown to produce substances capable of causing bone breakdown in vitro (7), and some of these substances appear to act independently of osteoclasts (8). Osteoclastic activity also appears to be increased by metastases (9), and osteoclastic activation has been proposed as the mechanism for production of bone lysis in myeloma (10). The excessive bone breakdown in patients with osseous metastases from breast cancer can be demonstrated by an increased urinary calcium and hydroxyproline excretion in some patients (6,11).

Mithramycin is an antibiotic with antitumor activity. It has been shown to have a marked inhibitory effect on bone breakdown in Paget's disease and myelomatosis as judged by urinary excretion of hydroxyproline and calcium (2,3). Minkin (12) showed that mithramycin inhibited bone resorption in cultured mouse calvaria and suggested that this might be due to the inhibition of formation of osteoclasts. Mithramycin is very effective in reducing the hypercalcemia which accompanies widespread metastasis in bone (13,14) and reduces urine hydroxyproline excretion in some patients (1). Although it is an ineffective agent against breast cancer and has marked hematologic toxicity when used in high doses, the drug produces its effects on bone breakdown in lower doses, when it is relatively safe.

For these reasons we treated the patients in this study with mithramycin to find out if it was effective in controlling severe pain and to gain experience with its long-term use in patients with widespread bone deposits. The response rates of bone metastases from breast cancer to combination chemotherapy are, in general, low, and we felt it necessary to use the drug as a single agent to see if healing of the bone would occur with mithramycin alone and because its long-term toxicity is unpredictable when it is used in combination.

Our patients had widespread, severe, and often incapacitating bone pain which was unresponsive to previous treatments. In one elderly patient (78 years old) systemic chemotherapy with cytotoxic drugs was thought inadvisable and mithramycin was used after hormone therapy produced no relief. Ten of 15 patients had relief of pain, and in seven the relief was considerable and rapid. Mobility was greatly improved in four patients. Relief of pain was most frequent in those patients in whom the treatment produced a decrease in alkaline phosphatase. This decrease occurred in eight patients. The plasma calcium fell slightly in all patients, but was especially marked in one patient who developed hypercalcemia in the period between mithramycin infusions. We did not note increased hydroxyproline excretion in our patients, a finding at variance with that of Cuschieri (6) and Powles et al (11). The reason for this discrepancy is not known.

Toxicity was tolerable, especially in the once-weekly regimen, and was confined to nausea which was usually mild. The weekly regimen has proved preferable to the 3-week regimen because it can be given on an outpatient basis; in two patients with hypercalcemia, weekly treatment produced a steady, sustained decrease in plasma calcium and alkaline phosphatase after the 3-week regimen had produced large variations in these values.

Chemotherapy for advanced breast cancer with combinations of cytotoxic drugs produces objective regressions in 40%–75% of patients (15,16), depending on the drugs used and the number and selection of patients. In general, response rates are highest in soft tissue lesions and lowest in bone metastases (17,18), although there is considerable difficulty in assessing the latter.

Mithramycin is an ineffective agent in the treatment of breast cancer, and did not produce healing of bone lesions, as determined by roentgenograms, and did not prevent the appearance of new lesions, as determined radiographically or on bone scans. For these reasons the drug cannot be recommended for use in the primary treatment of advanced breast cancer. It does, however, produce relief of pain in some patients and has proved safe and convenient to use, especially when given weekly. It should now be considered for use in the treatment of advanced breast cancer with bone metastases in combination with other agents which are effective against the tumor.

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Tamoxifen-Induced Tumor Stimulation and Withdrawal Response[1]

Sandra Legault-Poisson,* Jacques Jolivet, Roger Poisson, Mario Beretta-Piccoli, and Pierre R. Band[2]

SUMMARY

A postmenopausal breast cancer patient with a measurable lung metastasis was treated with tamoxifen. The tumor doubling time of the pulmonary lesion was 140 days during spontaneous growth and 52 days during tamoxifen therapy, an almost threefold increase in tumor growth rate. This stimulation persisted for 1 month after discontinuation of tamoxifen and was followed thereafter by a withdrawal regression lasting > 6 months. Awareness of the possible tumor growth enhancement with tamoxifen is of importance especially in view of its prolonged activity. Hormone withdrawal regression may occur following tamoxifen therapy, and a sufficient time interval should therefore elapse between cessation of tamoxifen and initiation of other therapeutic modalities.

[Cancer Treat Rep 63:1839–1841, 1979]

Tamoxifen, a synthetic triphenylethylene derivative, is of proven effectiveness in the treatment of hormonally sensitive metastases of postmenopausal breast cancer patients (1,2) and is currently being tested in adjuvant therapy studies in both premenopausal and postmenopausal patients. Although it generally produces minimal side effects (3), tamoxifen may cause temporary progression of skin lesions (4,5), exacerbation of tumor pain (3–7), and hypercalcemia (4,6,8). The occurrence of tumor growth enhancement of a measurable lung metastasis in a postmenopausal breast cancer patient treated with tamoxifen is the subject of this report.

CASE REPORT

A 50-year-old female experiencing menopause was first diagnosed as having an infiltrating ductal carcinoma of the right breast in October 1973. A simple mastectomy was performed in November of the same year, followed by irradiation therapy to the chest wall and lymph node drainage areas. Regular followup examinations with biannual chest roentgenograms and skeletal surveys were normal until May 1976, when a single lung metastasis appeared. The lung nodule slowly increased in size and chemotherapy[3] was started on January 31, 1977. After a period of stabilization, the tumor progressed and chemotherapy was stopped on September 14, 1977. On October 17, 1977, tamoxifen, 10 mg twice daily, was started; at that time, the patient was 5 years postmenopausal. A rapid increase in the size of the lung nodule occurred and tamoxifen was discontinued on February 9, 1978, after 110 days of treatment. No further therapy was given. One month later, continued progression of the lesion was still evident on a chest roentgenogram; however, the tumor later slowly decreased in size. At the time of this report, 6 months after discontinuation of tamoxifen, the tumor is still showing evidence of regression.

Standard chest roentgenograms were used to measure the diameter of the tumor nodule, in millimeters, at successive points in time, and the growth curve of this spherical lung metastasis during the various phases of its evolution was obtained (fig 1). The exponential nature of the curves, during spontaneous growth and during ta-

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[3] Sequential CMFP-AV as per protocol 3175 of the Eastern Cooperative Oncology Group. CMFP-AV = cyclophosphamide, methotrexate, 5-fluorouracil, prednisone, Adriamycin, and vincristine.

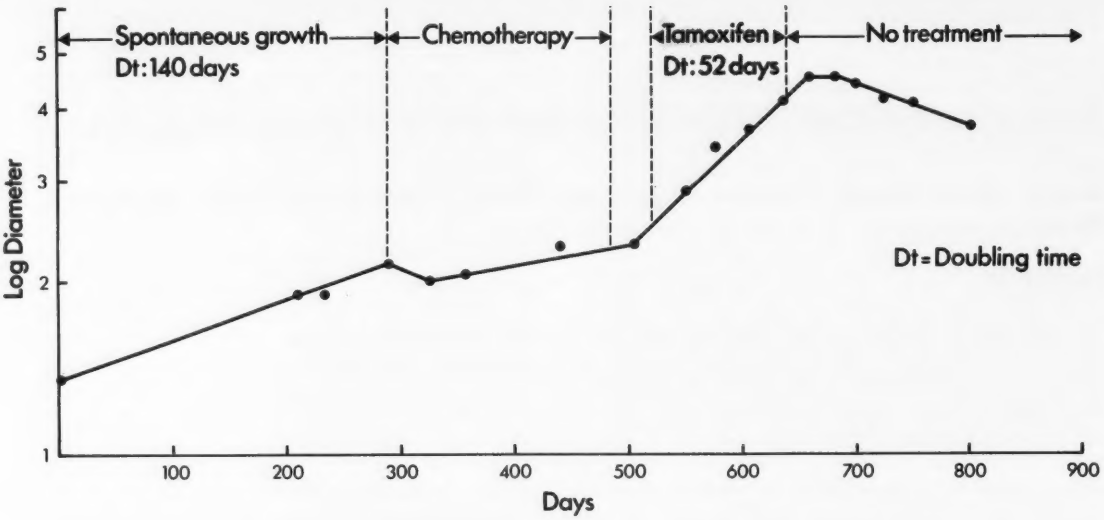

FIGURE 1.—Growth curve of a lung metastasis with modification of tumor doubling time at various phases of its evolution.

moxifen therapy, enabled calculation of the tumor doubling time (9,10). The tumor doubling time decreased from 140 days during spontaneous growth to 52 days during tamoxifen therapy, an almost threefold increase in tumor growth rate. Following cessation of tamoxifen, after a short period of persistent progression, a withdrawal regression became evident (fig 1).

Recently, we have also observed the occurrence of tamoxifen-induced tumor enhancement of prolonged duration in three postmenopausal breast cancer patients with osseous metastases. As opposed to tumor regression, which may follow transient bone pain exacerbation (3–6), all three patients experienced, shortly after the start of therapy with tamoxifen, marked and prolonged increase in bone pain associated with radiologically documented progression of lytic bone metastases.

DISCUSSION

In the patient presented, observation of the growth pattern of the lung metastasis (fig 1) suggests a hormonally sensitive tumor, as witnessed by the stimulation during tamoxifen therapy, and withdrawal regression upon discontinuation of the drug.

The study of tumor growth curves allows the objective assessment of response to therapy, since any therapeutic effect will be reflected in a change in the slope of the spontaneous growth curve (11). Israel and Chahinian have further demonstrated that upon early discontinuation of therapy or when resistance to treatment develops, the slope of the tumor growth curve becomes parallel to the spontaneous growth curve (12). Therefore, in our patient, tamoxifen induced definite tumor stimulation, as opposed to simple drug resistance, and its discontinuation provoked, in itself, a therapeutic regression.

Apparent paradoxes of hormonal treatment of breast cancer have recently been thoroughly discussed by Bruchovsky et al (13–15). These authors have reproduced, in experimental animal tumor systems, the various patterns of growth and regression occurring with hormonal manipulations. They have hypothesized that the growth of hormone-sensitive cells is regulated by three homeostatic constraint mechanisms termed initiation, negative feedback, and autophagia. During the process of malignant transformation, one or more of these processes may become inoperative. In the postmenopausal patient discussed, the pattern of response to tamoxifen, stimulation, and withdrawal regression suggests the persistence of functional initiation and autophagic mechanisms with deletion of negative feedback control.

The occurrence of tumor stimulation with estrogens is well-documented in breast cancer patients (16). The estrogenic activity of tamoxifen has been demonstrated in animal tumor systems; in mice, tamoxifen is fully estrogenic (17), whereas in rats it displays weak and atypical estrogenic properties (17,18). In man, estrogen-like properties of tamoxifen have been reported by some investigators (19); the tamoxifen-induced tumor exacerbations we have observed could possibly reflect a predominant estrogenic activity of the agent.

Tumor stimulation persisted for 1 month following cessation of tamoxifen in the case presented, and bone pain aggravation induced by tamoxifen also lasted for several weeks after drug discontinuation in the three patients with osseous metastases. The time lag observed between cessation of tamoxifen and disappearance of its adverse

manifestations reflects the agent's long-acting potential. Fromson et al have documented in animals (20) and in man (21) the long biologic half-life of tamoxifen, with prolonged biliary recirculation.

The withdrawal regression observed in our patient upon discontinuation of tamoxifen is noteworthy. Such an effect is known to occur after an initial response or failure to respond to estrogens (22), but has not been previously reported with tamoxifen. It is therefore suggested that, similar to what is recommended for estrogens, a sufficient time interval should elapse between cessation of tamoxifen and initiation of other forms of therapy, regardless of the initial response to tamoxifen.

As pointed out by Stoll (23), hormonal sensitivity is not always synonymous with tumor regression. Awareness of the possible stimulation of some patients with tamoxifen is of importance, especially in view of the prolonged activity of the agent.

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Initial Clinical Studies With Bruceantin [1,2]

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SUMMARY

A phase I clinical study of bruceantin was conducted in 66 patients with various types of advanced solid tumors to evaluate its toxicity and efficacy. The initial dose of 0.2 mg/m²/day × 5 days repeated at 2-week intervals was progressively increased to a maximum dose of 4.5 mg/m²/day. Hypotension was the dose-limiting toxic effect; it was delayed, cumulative, and occurred more often in patients with abnormal pretreatment liver function. Nausea, vomiting, and fever were common at higher doses, and diarrhea, stomatitis, alopecia, paresthesia, and rash were observed in some patients. The hematologic toxicity of bruceantin was moderate at high doses and was manifested mainly as thrombocytopenia; it was more severe in patients with abnormal hepatic and renal functions. No objective tumor regressions were observed. The recommended dose of bruceantin is 3.5 mg/m²/day × 5 days for phase II studies.

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Bruceantin is an alcohol extract isolated from the bark of *Brucea antidysenterica*, a simaroubaceous tree, that is used in Ethiopia to treat cancer patients (1). It has shown significant inhibitory activity against Walker 256 carcinosarcoma, P388 lymphocytic leukemia, L1210 lymphoid leukemia, Lewis lung carcinoma, and B16 melanoma (2). Its primary mode of action is through inhibition of protein synthesis (3). At a concentration of 5×10^{-9} M, bruceantin inhibits protein synthesis in HeLa cells by 90% and inhibits DNA synthesis by 40%, but shows little effect on RNA synthesis. This inhibitory action is not reversible in cell cultures (3). Preclinical toxicologic studies of bruceantin performed in dogs and monkeys disclosed significant acute gastrointestinal toxicity manifesting as anorexia, emesis, enteritis, hematochezia, melena, and hepatocellular damage. In addition, it was found to cause hematuria, azotemia, labored breathing, bradycardia, cardiac arrhythmia, hypothermia, and ataxia. In monkeys alone, bruceantin produced polypnea, muscle fasciculations, peeling of the lips, and dehydration. From preclinical experiments, it appeared that this compound had a significant antitumor effect. Hence, clinical studies were initiated in patients with advanced neoplastic disease. The purpose of these initial studies was to identify the maximally tolerated dose of bruceantin in man and to evaluate its antitumor activity against colorectal cancer and other tumors.

MATERIALS AND METHODS

Adult cancer patients admitted to the Department of Developmental Therapeutics were entered in this study. All patients had histologic proof of metastatic cancer and were no longer responding to conventional treatment of their tumors. Requirements for entry in the study included recovery from toxicity of previous therapy, normal blood cell counts, and normal renal and liver function tests. The starting dose of bruceantin was 0.2 mg/m²/day × 5 days. Subsequently, the dose of bruceantin was escalated by a factor of 100% if therapy was not associated with significant toxic effects. When toxic effects were observed, subsequent dose adjustments of 50% and 25% were performed. Initially, courses of therapy were repeated at 2-week intervals. Subsequently, they could

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TABLE 1.—Patient characteristics

Characteristic	
Total No. of patients	66
Males/females	45/21
Median age in yrs (range)	57 (28–74)
No. of patients with diagnosis of—	
Colorectal carcinoma	34
Lung carcinoma	18
Malignant melanoma	3
Sarcoma	2
Adenocarcinoma of unknown primary	2
Stomach cancer	2
Breast cancer	2
Other*	3

*1 patient each with lymphocytic lymphoma, Hodgkin's disease, and carcinoid.

only be repeated at 3- to 4-week intervals. The drug was dissolved in 250 ml of 5% dextrose solution and infused iv for 60 minutes. Upon completion of the phase I evaluation of bruceantin and establishment of its optimum dose level, a phase II evaluation in colorectal cancer was performed. All toxic effects noticed during or between courses of therapy were recorded.

Cbcs were performed twice weekly, while urinalysis and renal and liver function studies were repeated at least once weekly and prior to each course of therapy. Appropriate radiologic examinations as well as radioisotope-scanning procedures were usually performed prior to treatment and after two or three courses of treatment. Tumor measurements, recorded in centimeters, were the longest diameter and its perpendicular applied at the widest portion of the tumor. Lung lesions were measured by roentgenograms. A complete response was defined as the disappearance of all evidence of active tumor, with the patient free of symptoms for at least 8 weeks. A partial response was defined as a $\geq$ 50% decrease in the sum of the product of the largest diameters of the lesions. Stable disease was defined as no further growth of a previously rapidly growing tumor for at least 8 weeks or a response that was less than a partial remission. Progressive disease was defined as an unequivocal increase of at least 25% in the size of any measured lesion. The development of new lesions also constituted progressive disease.

RESULTS

A total of 66 patients received 173 courses of bruceantin; their characteristics are shown in table 1. All of the patients had previously received chemotherapy, usually more than one agent, and a number of these patients had been given radiation therapy. The dose levels of bruceantin ranged from 0.2 to 4.5 mg/m^2/day $\times$ 5 days in the patients in this study (table 2). Patients were entered at the initial dose level and subsequent dose escalations were made if no significant toxicity was seen. In most patients initial and subsequent therapy was given at dose levels of 3–4.5 mg/m^2/day.

Serial blood cell counts were obtained during 144 courses of therapy (table 3). Myelosuppression was infrequent at doses of < 3 mg/m^2, and courses of bruceantin at these doses could be repeated at 2-week intervals. At a dose of 3 mg/m^2, however, courses of treatment could not be repeated at 2-week intervals because of cumulative hematologic toxicity. During the first course, at this dose level, there was minimal myelosuppression as evidenced by the median lowest granulocyte and platelet counts of 2700 and 180,000/mm^3 respectively on Day 7 of therapy. However, the corresponding values with the subsequent course at the same dose level were 1200 and 80,000/mm^3 occurring on Days 20 and 21 of the therapy respectively. Because of cumulative hematologic toxicity at this dose level, the interval between the courses was increased to 3–4 weeks. The granulocyte and platelet counts at the 3-mg/m^2 dose level presented in table 3 summarize the overall result obtained at this dose level with the different dose schedules. Granulocytopenia at doses as high as 4.5 mg/m^2 was minimal; however, thrombocytopenia was moderate at the higher dose levels. The incidence of myelosuppression during courses of bruceantin was higher in patients with pre-existing hepatic (mainly increased bilirubin and alkaline phosphatase) or renal (increased BUN and creatinine) function abnormalities than in patients with no such abnormalities (table 4). Myelosuppression was generally brief, with recovery of all

TABLE 2.—Phase I dose escalation of bruceantin

Dose level (mg/m^2/day $\times$ 5)	Initial No. of—		Subsequent No. of—	
	Patients	Courses	Patients	Courses
0.2	4	7	—	—
0.4	4	6	3	3
0.8	4	7	3	4
1.5	3	6	7	9
3.0	23	33	5	16
3.6	7	14	3	8
4.5	21	39	13	21

TABLE 3.—Hematologic toxicity

Dose (mg/m^2/day × 5)	No. of evaluable courses	Neutrophil count: Median lowest (cells × $10^3/mm^3$) (range)	Day	% of courses ≤ 1000 cells	% of courses ≤ 500 cells	Platelet count: Median lowest (cells × $10^3/mm^3$) (range)	Day	% of courses ≤ 100,000 cells	% of courses ≤ 50,000 cells
0.2–1.5	38	4.8 (1.4–17.8)	8	—	—	253 (58–826)	9	5	—
3.0	44	2.4 (0.3–10)	13	7	2	150 (9–388)	14	25	14
3.6	16	2.8 (1.5–8.2)	12	—	—	161 (58–369)	12	6	—
4.5	46	2.6 (0.5–10.5)	14	6	1	178 (9–580)	14	25	6

TABLE 4.—Toxicity of bruceantin at dose levels of ≥ 3 mg/m^2 in relation to pre-existing liver and renal function abnormalities

Functional status: Liver	Functional status: Kidney	No. of evaluable courses	% of courses with—: Any toxic effects	% of courses with—: Hypotension*	% of courses with—: Myelosuppression†
Normal	Normal	89	60	20	10
Abnormal	Normal	14	93	50	43
Normal	Abnormal	14	78	—	43
Abnormal	Abnormal	5	100	20	60

*Defined as a drop in systolic pressure of ≥ 20 mm Hg and in diastolic pressure of ≥ 15 mm Hg.
†Defined as granulocyte count of ≤ 1000/mm^3 or platelet count of ≤ 100,000/mm^3.

TABLE 5.—Nonhematologic toxicity of bruceantin

Dose level (mg/m^2/day × 5)	No. of courses	No. of courses (%) with—: Any toxic effects	Vomiting	Hypotension*	Fever	Phlebitis
0.2–1.5	40	18 (45)	5 (13)	—	2 (5)	3 (8)
3.0	49	29 (59)	23 (47)	11 (22)	9 (18)	9 (18)
3.6	22	14 (64)	7 (32)	4 (18)	7 (32)	1 (5)
4.5	60	40 (67)	32 (53)	20 (33)	19 (32)	7 (12)

*Defined as a drop in systolic pressure of ≥ 20 mm Hg and in diastolic pressure of ≥ 15 mm Hg.

peripheral blood elements within 6 days. Cumulative myelosuppression did not occur among patients who received three or more courses of bruceantin at one dose level at intervals of ≥ 3 weeks.

A summary of the common nonhematologic toxicity of bruceantin is shown in table 5. Vomiting, hypotension, and fever were more common at doses of 3 mg/m^2 and increased in frequency with dose escalation. After the doses of bruceantin were administered, patients had a period of nausea lasting 3–4 hours, which was interrupted by one to three episodes of vomiting. Nausea and vomiting were more severe and prolonged when higher doses were administered. Hypotension, defined as a drop in systolic blood pressure of ≥ 20 mm Hg and in diastolic pressure of ≥ 15 mm Hg, was the dose-limiting toxic effect and occurred only at doses of ≥ 3 mg/m^2. A total of 35 episodes of hypotension occurred in 24 patients. During 31 courses of therapy, close monitoring of blood pressure before treatment and then hourly for 6 hours on the days chemotherapy was administered showed that the drop in blood pressure was greater during the 24 hours following the first daily dose of bruceantin than following doses of the 5-day course (table 6). The administration of bruceantin was discontinued following the second dose in two patients, the third dose in one patient, and the fourth dose in three patients, because of symptomatic hypotension which only occurred at doses of 4.5 mg/m^2. Hypotension occurred during the first treatment course of bruceantin in 19 patients, during the second course in 11 patients, and in subsequent courses in five patients.

TABLE 6.—Pattern of hypotension related to bruceantin at doses of ≥ 3 mg/m²/day × 5 days

Blood pressure reading*	Mean blood pressure during treatment on Day—				
	1	2	3	4	5
Immediately before therapy	132/84	108/69	105/67	108/69	106/68
Lowest blood pressure from start to 3 hrs after completion of treatment	130/83	110/70	102/65	100/60	108/68
(No. of patients removed from study because of hypotension)		(2)	(1)	(3)	

*Blood pressure in mm Hg = systolic/diastolic.

Eight episodes of hypotension occurring in seven patients were associated with dizziness (three), confusion (two), hemiparesis (two), slurred speech (two), headache (two), azotemia (two), cranial nerve paresis (one), and hoarseness of voice (one). These symptoms, except for residual weakness and renal failure, each occurring in one patient, disappeared upon discontinuation of bruceantin and initiation of supportive care. Mixing the daily dose of bruceantin in 500 ml of 5% dextrose in water (instead of 250 ml) and prolonging the duration of administration to 3–4 hours did not seem to alter significantly the frequency and severity of episodes of hypotension.

Febrile reactions to bruceantin occurred during one third of the courses at doses of > 3 mg/m². The temperature elevation was mild, ranging between 100°F and 101°F; it occurred within 4 hours after completion of bruceantin administration and was accompanied by chills in one third of the patients. Phlebitis was less common and was manifested as a burning sensation for 3–4 hours, followed by the appearance of a red streak along the vein used for infusion in 24 hours. The frequency and severity of phlebitis were reduced by diluting the daily dose of bruceantin in 500 ml of fluid and prolonging the time of administration to 4 hours. Other less frequent toxic effects observed during 171 evaluable courses of bruceantin included diarrhea (16 courses), abdominal cramps (nine), anorexia (eight), stomatitis (seven), alopecia (five), headache (five), paresthesia (four), maculopapular rash (four), and azotemia (three).

Although all of the 66 patients were evaluable for immediate toxicity, two died within 1 week of starting bruceantin treatment, two returned home immediately following completion of their first course of treatment and refused further followup, and an additional four were removed from the study before completion of the first bruceantin treatment course because of severe hypotension and were not evaluable for response. The remaining 58 evaluable patients included 24 with colorectal carcinoma who received bruceantin at dose levels ≥ 3 mg/m². No objective responses were seen. Stable disease lasting for > 8 weeks occurred in 15 patients at doses of ≥ 3 mg/m². This included seven patients with colorectal cancer, two with adenocarcinoma of the lung, two with malignant melanoma, and one each with squamous cell carcinoma of the lung, carcinoid, lymphocytic lymphoma, and adenocarcinoma of unknown primary. The remaining patients had tumor progression.

DISCUSSION

This phase I evaluation of bruceantin, administered as a 5-day iv course, demonstrated that the optimum dose of bruceantin in a 5-day schedule is 3.6 mg/m²/day. When the drug is given frequently, the interval between courses should be 3 weeks because of cumulative myelotoxicity. The most important side effect observed at this dose level was asymptomatic hypotension which occurred during four of 22 courses. The incidence and severity of hypotension and other toxic effects of bruceantin therapy increased substantially at doses of > 3.6 mg/m². Eight of 22 (40%) episodes of hypotension at the 4.5-mg/m² dose level were associated with symptoms related to cerebral and renal vascular insufficiency. Episodes of hypotension due to bruceantin, although dose-related, were unpredictable. Five of 14 patients who received a course of bruceantin at the ≥ 3-mg/m² dose level, following an episode of hypotension, had recurrence of hypotension, which resulted in neurologic and renal complications in two. Hypotension was more frequent in patients with hepatic function abnormalities. Although thrombocytopenia (platelet count ≤ 100,000/mm³) occurred in only 6% of the courses given at the 3.6-mg/m² dose level, it occurred in 25% of the courses given at the 4.5-mg/m² dose level. Severe thrombocytopenia (platelet count ≤ 50,000/mm³) occurred in 6% of the courses given at 4.5 mg/m². The patients who had excessive myelosuppression generally had received prior radiotherapy to the pelvis and spine, had hepatic or renal dysfunction, or had received prior chemotherapy with drugs such as nitrosoureas.

On the basis of these observations, we recommend a starting dose of 3.5 mg/m²/day × 5 days of bruceantin for phase II studies in patients with normal pretreatment

hepatic and renal functions. For poor-risk patients, ie, those with abnormal pretreatment hepatic and renal functions and those with extensive prior radiotherapy and chemotherapy, the starting dose should be in the range of 2.5–3 mg/m^2. Since hypotension rather than myelosuppression was the dose-limiting toxic effect of bruceantin, and routine blood cell counts will not reflect early bruceantin toxicity, its use, like that of ftorafur, vincristine, bleomycin, streptozotocin, and maytansine, will require special precautions. Close monitoring of blood pressure, both before treatment and then hourly for 6 hours on the days bruceantin is administered, is indicated. Should a significant drop in blood pressure occur, the administration of bruceantin must be discontinued and supportive treatment must be initiated.

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Phase I Clinical Evaluation of 2,3-Dihydro-1*H*-imidazo[1,2-*b*]pyrazole [1,2]

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SUMMARY

2,3-Dihydro-1*H*-imidazo[1,2-*b*]pyrazole, a DNA synthesis inhibitor, was given to 25 patients in a phase I study. The drug was administered by rapid iv infusion daily × 5 days at 3-week intervals at doses ranging from 150 to 1500 mg/m^2/day. Side effects were observed with doses of ≥ 1000 mg/m^2/day and included nausea and vomiting, diarrhea, dark urine, and anemia. At doses of 1500 mg/m^2, three patients had evidence of hemolysis (two had hemoglobinuria and one had acute intravascular hemolysis). The hemolysis was severe enough to cause death in one patient and necessitated abandoning further dose escalation. There was minimal or no myelosuppression at any dose level. No objective tumor regression was observed in any of the 16 patients evaluable for response. Further studies are recommended to carefully evaluate the etiology of the hemolysis before proceeding to a phase II trial. It is unlikely that this drug will prove to be useful unless methods for circumventing hemolysis are developed.

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2,3-Dihydro-1*H*-imidazo[1,2-*b*]pyrazole (IMPY) is a potent DNA synthesis inhibitor, which does not inhibit RNA or protein synthesis (1). The inhibition of DNA synthesis is rapidly reversible, and in HeLa cells there is also synchronization of cell cycle at the point of entry in the S phase (1,2). IMPY was selected for further development because of demonstrated activity against mouse L1210 leukemia and L1210 variants made resistant to the imidazole, DTIC, and the imidazole mustard, BIC. It is also active against L1210 resistant to cytosine arabinoside and thiosemicarbazone. In addition, the combination of optimal schedules of IMPY and DTIC has shown superiority over that of each drug alone against the L1210 leukemia system. The drug is only marginally active against Ridgway osteogenic sarcoma, 755 adenocarcinoma, and B16 melanoma, and it is completely inactive against P388 leukemia. Recently, it has been suggested that a metabolite of IMPY may be responsible for the observed persistent inhibition of DNA synthesis in vivo, for the antileukemic activity, and for the observed lack of schedule dependency (3).

Animal toxicology studies conducted in beagle dogs and rhesus monkeys demonstrated that the major target organs affected by IMPY included the liver, kidneys, lymphoid tissue, bone marrow, and gastrointestinal tract. Dose-related changes observed included anemia, reticulocytosis, leukopenia, leukocytosis, increased creatinine and BUN, SGOT, SGPT, alkaline phosphatase, bilirubin, and BSP retention. Clinical manifestations of drug toxicity included anorexia, adipsia, vomiting, dark urine (due to the presence of hemoglobin or bilirubin), melenic diarrhea, hypothermia, mydriasis, tremors, tachycardia, dyspnea, weight loss, hypoactivity, ataxia, and brief lapses of consciousness. The immediate toxic effects were on the rbcs, resulting in hemolysis with suppression of lymphoid tissues occurring later. Pathologically, there was lymphoid hypoplasia, necrosis, and atrophy of the lymph nodes and spleen. Toxic changes, including congestion and necrosis, were also seen in the liver and gastrointestinal tract.

The purpose of this study was to determine the maximum-tolerated dose and toxicity associated with the

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administration of this compound. A daily × 5 day intermittent schedule of drug administration was selected since marked schedule dependency was not seen in the L1210 system. The starting dose recommended was based on the toxicity observed with IMPY in experimental animals. The monkey was the more sensitive animal and the toxic dose low was 498 mg/m² for a daily × 5 day dose administration schedule. One third of this dose, approximately 150 mg/m², was the initial dose chosen for our phase I clinical trial in man.

MATERIALS AND METHODS

All adult patients with histologic proof of malignancy, who were not candidates for known regimens or protocol treatments of higher efficacy or priority, were considered eligible for the study. An estimated life expectancy of at least 12 weeks was required, while the presence of measurable lesions was not a criterion. Patients were required to have had all previous chemotherapy, radiotherapy, or immunotherapy discontinued for at least 4 weeks prior to being entered in the study, with recovery from the toxic effects of that therapy. All patients were required to have adequate bone marrow function (defined as a peripheral absolute granulocyte count of > 2000/mm³ and a platelet count of > 100,000/mm³ with normal bone marrow cellularity), adequate liver function (bilirubin < 2 mg/100 dl), and adequate renal function (creatinine < 2.5 mg/100 ml). Informed consent indicating the investigational nature of the study was obtained from all patients according to institutional policy.

IMPY was supplied in 20-ml amber vials containing 500 mg as a water-soluble white crystalline solid. For clinical use, IMPY was reconstituted with 10 ml of sterile water for injection (USP) and was further diluted in 50 ml of 5% dextrose in water to be given iv over 30–60 minutes. The treatment schedule was daily × 5 days repeated every 3 weeks. The starting dose of IMPY was 150 mg/m²/day × 5 days, and dose escalations of 50%–100% were made until there was evidence of toxicity. At least three patients were studied at each dose level and were evaluated for at least 3 weeks before additional patients were given the next dose level. Initially, to avoid cumulative toxicity, no dose escalation was permitted for a patient until further observations indicated that the time course selected for repeated administration was satisfactory.

Complete medical histories and physical examinations were performed prior to treatment. Cbc with differential and platelet counts, SMA-12 chemistry battery screen, and urinalysis were repeated at least once each week and prior to each course of therapy. Appropriate radiologic examinations, as well as radioscanning procedures, were performed as clinically indicated before and during treatment. Treatment was discontinued when there was evidence of tumor progression following one or more courses of treatment. Patients were considered evaluable for toxicity if they received at least one course of therapy with 3 weeks of subsequent observation. A partial response was defined as a ≥ 50% decrease in the sum of the products of the diameters of all measured lesions. Stable disease was defined as a response which was less than a partial response or no further increase of a previously rapidly growing tumor. Progressive disease was defined as an increase by at least 50% in the size of any measured lesion or the development of new lesions.

TABLE 1.—Patient characteristics

Total No. of patients	25
Age in yrs	
Median	52
Range	34–66
Sex	
Males	19
Females	6
No. of patients with diagnosis of—	
Malignant melanoma	8
Adenocarcinoma, colon	6
Adenocarcinoma, lung	4
Squamous cell carcinoma, lung	3
Sarcoma	2
Adenocarcinoma, unknown primary	1
Squamous cell carcinoma, skin	1

RESULTS

Twenty-five cancer patients received IMPY at doses ranging from 150 to 1500 mg/m²/day × 5 days. Patient characteristics are shown in table 1. A total of 51 treatment courses were administered; the dose escalation scheme is shown in table 2.

All 25 patients were evaluable for acute toxicity. However, two patients died of progressive disease within 1 week after starting treatment (at 450- and 1000-mg/m² dose levels, respectively), and one patient refused further treatment after the first course of therapy at 450 mg/m²/day × 5 days. An additional patient had an acute onset of chest pain on Day 3 of IMPY at a dose of 1500 mg/m²/day associated with bradycardia, atrioventricular disso-

TABLE 2.—IMPY: dose escalation scheme

Dose (mg/m²/day × 5)	No. of patients	No. of courses		
		Initial	Subsequent	Total
150	5	5	3	8
300	6	5	7	12
450	8	5	6	11
1000	5	5	4	9
1500	6	5	6	11

TABLE 3.—Hematologic toxicity*

Dose level (mg/m^2/day × 5)	Median counts (cells × $10^3/mm^3$)			Median hemoglobin (g/100 ml)
	Wbcs	Neutrophils	Platelets	
	Lowest (range)/Day	Lowest (range)/Day	Lowest (range)/Day	Lowest (range)/Day
150	6.6(4.4–14.6)/10	4.1(3.3–13.0)/10	171(67–302)/11	11.0(7.9–11.8)/4
300	5.2(4.4–9.6)/14	3.6(2.6–7.0)/14	234(110–345)/14	10.1(9.9–14.1)/11
450	4.8(3.6–12.1)/11	3.3(2.4–8.5)/11	286(137–493)/11	10.9(8.5–13.5)/10
1000	3.9(2.6–13.8)/15	2.1(1.3–11.7)/15	148(89–266)/14	12.3(10.0–14.0)/8
1500	5.5(2.3–8.9)/21	3.8(1.8–6.9)/21	154(103–465)/10	8.6(3.9–10.2)/8

*Represents all episodes of myelosuppression observed during therapy with IMPY at each dose level.

ciation, and hypothermia. There was, however, no enzyme or ECG evidence of myocardial infarction. IMPY was immediately discontinued. No drug-related toxic effects were observed in any patient who had received < 1000 mg/m^2/day × 5 days of IMPY. Of the five patients treated at the 1000-mg/m^2 dose level, two had nausea and vomiting and one had diarrhea and dark urine. The etiology of the dark urine was unclear since all tests for hemolysis (including urine hemoglobin and bilirubin, Coombs' test, and haptoglobin) were negative. A drop in the hemoglobin level of > 2 g/dl was observed in 30% of courses given at the 1000-mg/m^2/day dose level. This drop usually occurred over a 2-week period and the hemoglobin would return to normal by Day 22. Significant toxicity was observed at a daily dose of 1500 mg/m^2/day × 5 days. Of the six patients treated at this dose level, three had nausea and vomiting, one had diarrhea, and three had dark urine. The urine was positive for hemoglobin in two patients. An additional patient, who was a 48-year-old black male with adenocarcinoma of the lung, developed evidence of severe hemolysis after his third dose of IMPY, with the hemoglobin level dropping from 8.7 to 3.9 g/dl; haptoglobin, < 41 units; serum potassium, 6.5 mEq/liter; total bilirubin, 8.2 mg/100 ml; and urine positive for bilirubin but negative for hemoglobin. The patient died the following day of complications resulting from hypotension related to hemolysis, in the presence of far-advanced cancer. During 75% of courses given at the 1500-mg/m^2/day level, the patients experienced a drop in the hemoglobin level of > 2 g/dl occurring over a 2-week period (except for the patient with acute hemolysis) and recovering by Day 22. Otherwise, there was minimal or no myelosuppression at any dose level (table 3).

No objective tumor regression was observed in any of the 16 patients evaluable for response. However, one patient with squamous cell carcinoma of the skin metastatic to the lungs had 10%–20% regression of his lung nodules which stabilized for at least 3 months with treatment continued at the 300-mg/m^2/day dose level. Four other patients had stable disease: three with metastatic melanoma and one with adenocarcinoma of the colon.

DISCUSSION

In this phase I study of IMPY, further dose escalations of > 1500 mg/m^2/day × 5 days were prevented by the frequent occurrence of hemolysis, although no other significant biologic effects or antitumor activity was observed at this dose. The hemolysis was severe enough to cause death in one patient and necessitated abandoning further dose escalation. The etiology of the hemolysis is not clear. However, it has been reported that IMPY causes oxygen-dependent stimulation of the hexose monophosphate shunt associated with hydrogen peroxide production, which may explain the hemolysis (4).

No definite conclusions can be made regarding the antitumor activity of IMPY since only a relatively small number of patients were studied. The lack of myelosuppression would make it an attractive drug for combination chemotherapy if it is found to have some antitumor activity in further clinical studies. However, before IMPY can be recommended for a phase II trial, further studies are clearly needed to carefully evaluate the etiology of the hemolysis. Clinical pharmacology studies and tests of different schedules of administration are also required. It is unlikely that this drug will prove to be useful unless the cause of the hemolysis can be clarified and methods for circumventing the hemolysis can be developed.

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Treatment of Acute Nonlymphocytic Leukemia With Neocarzinostatin [1,2]

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SUMMARY

Neocarzinostatin (NCS) is a polypeptide antibiotic which has been shown to have antileukemic activity in Japanese trials. Twenty-two patients, who were in relapse with acute nonlymphocytic leukemia refractory to other agents, were studied with two dose schedules of NCS. Schedule 1, 2000–8000 units/m^2/day by continuous infusion for 5–10 days, was given to 12 patients and schedule 2, 2000–6000 units/m^2/day by iv bolus every 8 hours, was given to ten patients. Clearing of peripheral blast cells was seen in nine of 12 patients treated with schedule 1 and in four of ten patients treated with schedule 2. Bone marrow effect was variable and appeared to be dose-related. No unequivocal complete remissions were seen with either schedule. NCS perturbations of cycling bone marrow cells were studied in two patients receiving the drug by continuous infusion. Both of these patients showed G_2 accumulation of marrow cells following treatment. Immunochemically detectable levels of drug were seen in three patients treated with continuous infusion. In summary, NCS has antileukemic effect in patients with acute nonlymphocytic leukemia refractory to other agents. However, its role in the therapy for acute leukemia requires further evaluation.

[Cancer Treat Rep 63:1853–1856, 1979]

Neocarzinostatin (NCS) is a polypeptide antibiotic isolated from the culture filtrate of *Streptomyces carzinostaticus* (1). The drug is an acidic protein macromolecule of molecular weight 10,700 consisting of a single polypeptide chain of 109 amino acids cross-linked by two disulfide bridges (2). NCS is strongly active in a number of animal tumor systems, including SN36 (3) and L1210 (4–6) leukemias.

NCS has undergone extensive clinical trial in acute leukemia in Japan. It has been administered alone or in combination with other agents active in leukemia. Although most patients receiving NCS had had extensive prior treatment, the drug appeared to have some degree of leukemocidal activity in the majority of patients. Single-agent complete response rates of 40%–50% in previously untreated patients have been reported (7–10).

In view of the lack of cross-resistance with the clinically available antileukemic drugs and clinical activity in previously treated patients, a cooperative clinical trial of NCS in acute nonlymphocytic leukemias in relapse was performed at the Sidney Farber Cancer Institute and Peter Bent Brigham Hospital in Boston and at the St. Bartholomew's Hospital in London.

MATERIALS AND METHODS

NCS was provided by the National Cancer Institute (Bethesda, MD) and was obtained from Kayaku Antibiotics Research Ltd (Tokyo, Japan). The drug was supplied in 2-ml vials containing 2000 units of NCS in sodium acetate-acetic acid buffer (0.015 M, pH 5.0). For the pur-

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TABLE 1.—Dose and schedule of NCS in 22 patients

	No. of courses	No. of patients
Schedule 1 (12 patients): continuous infusion for 5, 7, or 10 days		
Dose (units/m²/day)		
2000	2	1
4000	2	2
6000	12	10
8000	2	2
Schedule 2 (10 patients): iv bolus q8hrs for 5 days		
Dose (units/m²/day)		
2000	13	6
6000	4	4

poses of comparison to Japanese drug regimens, 1400 units is considered to be equivalent to 1 mg.

Two dose schedules were employed (table 1). Twelve patients received continuous infusion of the drug, at the stated dose levels, for periods of 5, 7, or 10 days. The majority of patients received 6000 units/m²/day for 5 or 7 days. The other ten patients received one third of the total daily dose as an iv bolus repeated every 8 hours for 5 days. Courses were repeated at 2–3-week intervals, depending upon the clinical response.

Patient characteristics are presented in table 2. All patients had received intensive prior treatment with an anthracycline antibiotic, cytosine arabinoside, and other drugs. Six patients had never obtained a complete remission and the other 16 had relapsed from their remissions and became resistant to their initial therapies. The median survival from diagnosis to entry in the study was 92 weeks. All patients studied had severe depression of normal myelopoiesis secondary to their disease and previous treatment, and the majority required continuous platelet transfusion support. By morphologic criteria, these 22 patients had disease characterized as acute myelogenous leukemia (15 patients), acute myelomonocytic leukemia (two), erythroleukemia (one), blast cell crisis of chronic granulocytic leukemia (one), acute monocytic leukemia (one), acute promyelocytic leukemia (one), and myeloblastoma-acute myelocytic leukemia (one).

Radioimmunoassay was performed by a double-antibody technique, as previously described (11). Serum samples (5 ml) were obtained from the arm opposite the site of infusion 48 hours after the start of continuous infusion of drug. Samples were stored at –20°C until assayed.

Bone marrow samples (1 ml) were obtained in two patients by iliac crest aspiration, immediately before and 48 hours after the start of continuous-infusion NCS. DNA distribution histograms were obtained by a previously described cytofluorometric method (12,13). Concurrent morphologic analysis of these specimens showed 90% replacement of normal marrow by leukemic cells. A third marrow aspirate was obtained in one patient 7 days after the start of infusion.

TABLE 2.—Patient characteristics

Characteristic	
Total No. of patients	22
Sex (M/F)	11/11
Median age in yrs (range)	41 (18–64)
Median No. of previous remissions (range)	1.0 (0–3)
Median survival in wks from diagnosis to entry in study (range)	92 (3–172)
No. of patients not previously in remission	6

RESULTS

Toxicity.—Immediate toxic effects were minor and were not dose-limiting with either schedule of administration. Gastrointestinal toxicity was typically manifested by anorexia and nausea, without frank vomiting. Multiple episodes of minor extravasation occurred with the continuous-infusion schedule; these led to local erythema and pain, but no ulceration occurred. Minor temperature elevation (to 100°F) was seen following drug administration with the iv bolus schedule. No allergic reactions were seen with either dose schedule.

Antileukemic effect.—The effect on circulating blood elements was immediate and pronounced. Leukemocidal activity, as evidenced by a fall in the blast cell count, was seen in all patients. The nadir day for blast cells was early (median, Day 8; range, Days 0–22). The median pretreatment peripheral blast cell count was 8000/mm³ (range, 0–291,000/mm³) and the median posttreatment value was 0/mm³ (range, 0–5400/mm³), reflecting that the majority of patients showed complete clearing of peripheral blast cells. The paucity of normal blood elements in these patients made the quantification of drug effect on normal myelopoiesis difficult. However, patients in the study remained leukopenic and thrombocytopenic throughout the period of treatment, and several demonstrated increased transfusion requirements for platelets. Complete clearing of peripheral blast cells was seen in nine of 12 evaluable patients and 13 of 16 evaluable courses among patients treated with continuous-infusion NCS and in four of ten patients and six of 17 courses among patients treated with iv bolus NCS. Bone marrow examinations were obtained before and after treatment in eight patients receiving continuous-infusion NCS; six of these patients showed marked hypoplasia of all elements, both normal and leukemic. Similar hypoplasia was seen in two of six serial marrows obtained in the schedule 2 patients (table 3). On five occasions, repeat bone marrow examinations were not obtained despite clearing of peripheral blast cells. These patients were extremely pancytopenic and further therapy was not considered appropriate at that time. There were no complete remissions (CRs) that could be unequivocally attributed to

TABLE 3.—Antileukemic effect of NCS

NCS treatment	Clearing of peripheral blast cells: No. of evaluable patients/No. treated	Clearing of peripheral blast cells: No. of evaluable courses/No. given	No. of patients with marrow hypocellularity/ No. evaluable	No. of patients with CR/No. evaluable
Schedule 1: continuous infusion	9/12	13/16	6/8	1/5
Schedule 2: iv bolus q8hrs	4/10	6/17	2/6	0/5

NCS treatment. One CR occurred in a patient who had received treatment with an anthracycline-cytosine arabinoside regimen 16 days prior to receiving NCS, without response. The patient then received two courses of NCS at the lowest dose employed in the study, 2000 units/m^2 for 5 days, and entered CR on Day 76 after the start of NCS. This remission persisted unmaintained for 6 months, and upon relapse, re-induction with single-agent NCS was unsuccessful.

Radioimmunoassay.—Immunochemically detectable levels of NCS were seen at all dose levels employed in the study. Three patients were studied, one at each dose level. The values obtained at 2000, 4000, and 6000 units/m^2 were 40, 180, and 240 10^{-10} mols/liter, respectively.

Cytofluorographic studies.—Perturbations of cycling leukemic cells in bone marrow were examined in two patients by cytofluorography, and the results are shown in figure 1. Both patients showed increased numbers of cycling cells in the G_2 portion of the cell cycle after NCS infusion, compared to pretreatment cytofluorographic studies. Subsequent study of the first patient 7 days later showed persistent, but less marked, G_2 accumulation of cycling cells.

DISCUSSION

Japanese investigators have reported significant activity of NCS in acute leukemia. These reports have been reviewed by Legha et al (7). An analysis of 76 patients with acute leukemia, predominantly acute nonlympho-

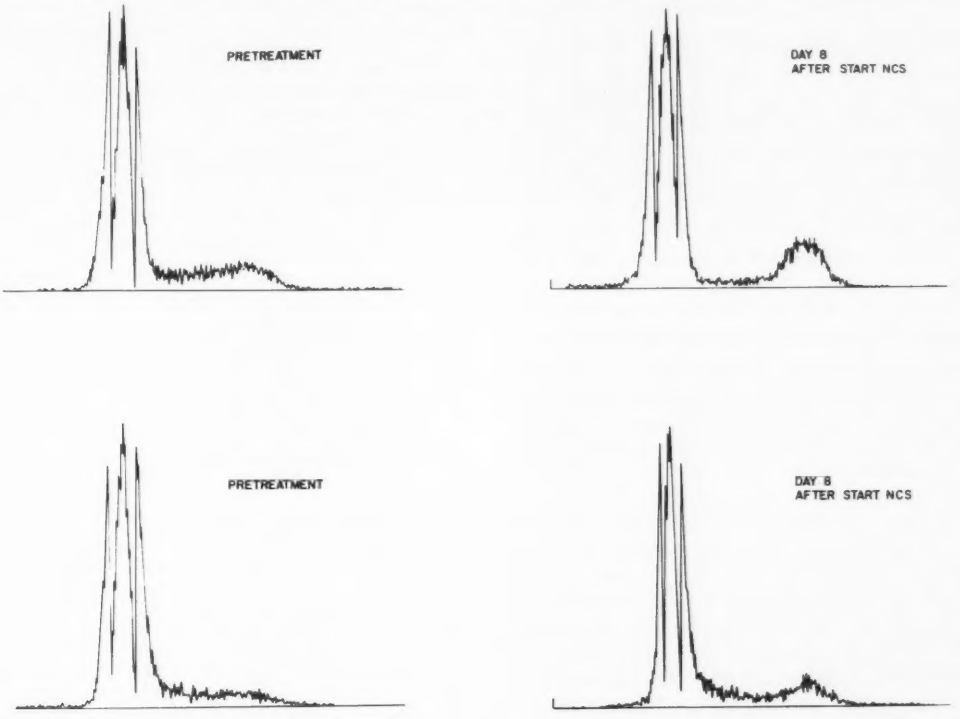

FIGURE 1.—Results of cytofluorographic studies of leukemic cells in bone marrow in 2 patients treated with NCS.

cytic leukemia, treated with single-agent NCS at eight institutions demonstrated a CR rate of 13%. It is of note that seven of these 11 CRs occurred in a group of 18 previously untreated patients at one institution.

The American experience has been less encouraging, perhaps reflecting the more advanced stage of leukemia treated in the American studies. A variety of doses and dose schedules have been employed. Ohnuma et al (14) reported one CR, lasting 3 months, in a group of nine patients with acute leukemia treated with single-agent NCS. Doses employed ranged from 2000 to 12,000 units/m^2/day × 5 days, with the drug being administered as a continuous infusion. Van Echo and Wiernik (15) treated 14 adult patients with refractory acute nonlymphocytic leukemia with NCS, at doses ranging from 1000 units/m^2/day × 5 days to 3500 units/m^2/day × 15 days, with the drug being administered as an infusion over 30–90 minutes. Marrow hypoplasia was produced at the higher dose levels, but no CRs were seen. McKelvey et al (16) have reported two CRs and two partial remissions in a group of patients with leukemia (acute myelocytic leukemia [12 patients], acute lymphocytic leukemia [seven], and chronic lymphocytic leukemia [five]) treated with a continuous or intermittent iv infusion of NCS in doses ranging from 1000 to 6500 units/m^2/day × 5. Greater clinical improvement was seen with the intermittent-infusion schedule (16).

This present study demonstrates that NCS possesses a clinical antileukemic effect in patients resistant to conventional agents. The increased antileukemic effect seen in schedule 1 patients was most likely related to the higher dose received by the schedule 1 patient (mean dose per patient was 5700 units/m^2/day, compared to 3700 units/m^2/day for schedule 2 patients) rather than to the difference in scheduling. At the higher dose levels, complete marrow hypoplasia could be produced, with little or no nonmyelosuppressive toxicity. Therefore, NCS may be a useful agent in producing bone marrow aplasia prior to heterologous or autologous bone marrow infusion.

In summary, single-agent NCS, in the dose schedules employed, produced little clinical benefit in these end-stage patients with acute leukemia. Further evaluation of this agent in the treatment of acute leukemia may require determination of optimal schedule and treatment of patients at an earlier stage of disease.

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Antitumor Activity of Pyrazofurin in Combination With 5-Azacytidine Against Murine P388 and L1210 Leukemias and Colon Carcinoma 26[1,2]

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SUMMARY

The antitumor activity of the sequential combination of pyrazofurin (PF) followed by 5-azacytidine (5ACR) was evaluated in two murine ascitic leukemias, P388 and L1210, and in murine colon carcinoma 26. Both the drug and the tumor were administered ip. Use of this combination was based on the rationale that treatment with PF, an inhibitor of de novo pyrimidine nucleotide biosynthesis, would provide a synergistic effect with 5ACR due to a relative increased incorporation of 5ACR nucleotides. PF alone has marginal activity against the leukemias, evident only at a maximally tolerated dose. 5ACR was quite effective against the murine leukemias, both when treatment was initiated on Day 1 and when treatment was delayed until 4 days after tumor inoculation. The combination of PF given 6 hours before 5ACR had greater than additive host toxicity. Suboptimal doses of 5ACR, with little or no antileukemic effects when used alone, were highly effective with PF pretreatment. In early L1210, advanced L1210, and advanced P388 leukemias, the combination of PF and 5ACR produced significantly greater prolongation of lifespan than could be achieved with a maximally tolerated dose of 5ACR alone. In animals bearing ip colon carcinoma 26, the combination of PF and 5ACR was also moderately active, whereas both drugs were inactive when used alone. The results of these therapeutic studies support the finding from in vitro studies that PF pretreatment enhances the cytotoxic activity of 5ACR. However, enhanced host toxicity suggests that clinical trials of this combination should employ low doses of the drugs with cautious escalation of the 5ACR dose while keeping the dose of PF constant.

[Cancer Treat Rep 63:1857–1862, 1979]

Pyrazofurin (PF), a C-riboside isolated from a strain of *Streptomyces candidus* (1,2), exhibits antiviral and antitumor activity (1,3). PF has significant antitumor activity in several rodent tumors, including Walker 256 carcinosarcoma, 755 mammary adenocarcinoma, Gardner lymphosarcoma, X5563 plasma cell tumor, and DMBA-induced mammary carcinoma (3). PF, when activated to the 5-monophosphate by adenosine kinase, is a potent inhibitor of orotidylate decarboxylase, and thus blocks de novo pyrimidine nucleotide biosynthesis (4,5). Initial phase I clinical trials with PF have indicated dose-limiting toxic effects at doses of 100–300 mg/m^2/week, 250 mg/m^2/24 hours (6), or 45 mg/m^2/day × 5 every 2–3 weeks (7). Major toxic effects were mainly mucosal and cutaneous manifestations, and, to a lesser extent, hematologic toxicity. Rare antitumor effects were seen in acute myelogenous leukemia, Hodgkin's disease, and breast carcinoma

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(5–8). In a phase II study of PF in previously untreated metastatic colorectal adenocarcinoma, no responses were seen in 35 patients treated with PF alone at doses of 240 mg/m^2 every 3–4 weeks (9).

The pyrimidine nucleoside analog 5-azacytidine (5ACR) exerts its antitumor activity through incorporation into both DNA (10) and RNA (11) with subsequent inhibition of protein biosynthesis (12). It has demonstrated promising activity in patients with acute myelogenous leukemia who had become refractory to conventional chemotherapy regimens (13–15). Its major toxic effects have been gastrointestinal (nausea and vomiting), hematologic, and hepatic (13). Cutaneous toxicity was observed in only 2% of the patients (13).

The possible synergistic effect of the sequential combination of PF followed by 5ACR has been suggested by the in vitro studies of Cadman et al (16) and Plagemann et al (17). These studies were based on the rationale that depletion of uridine and cytidine nucleotide pools caused by PF would lead to enhanced uridine-cytidine kinase activity, and hence to increased phosphorylation of 5ACR administered subsequently. Increased uptake of 5ACR by murine and human leukemia cells and Novikoff hepatoma cells in vitro was in fact observed following PF treatment (16,17). The aim of the present study was to evaluate the potential synergistic antitumor activity of this combination in vivo. The combination was studied in mice bearing P388 and L1210 leukemias, tumors which are quite sensitive to 5ACR but relatively refractory to PF, a situation possibly analogous to human myelogenous leukemia. PF followed by 5ACR was also evaluated in mice bearing colon carcinoma 26, a solid tumor that is refractory to both drugs when used alone.

MATERIALS AND METHODS

Drugs.—PF was generously supplied by Eli Lilly and Co (Indianapolis, IN). 5ACR was obtained from the Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). Both drugs were dissolved in 0.9% NaCl solution and administered ip in a volume of 0.01 ml/g of body weight. Solutions of 5ACR were constituted immediately prior to use.

Mice.—Male BALB/c × DBA/2 (hereafter called $CD2F_1$) and C57BL/6 × DBA/2 (hereafter called $B6D2F_1$) mice, 6–8 weeks of age and weighing 18–24 g, were used in these studies. Mice were housed in groups of eight in plastic cages, and were supplied with tap water and Purina laboratory chow ad libitum.

Tumors and chemotherapy studies.—P388 leukemia, L1210 leukemia, and colon carcinoma 26 were maintained by serial transplantation in syngeneic mice. The leukemias were implanted in $B6D2F_1$ mice by ip inoculation of 10^6 viable cells according to standard protocols of the National Cancer Institute (18). Colon carcinoma 26 was inoculated ip as a 1% tumor brei in $CD2F_1$ mice, as previously described (19). Drug treatment was initiated either 1 day or 4 days after tumor implantation and was given every 4 days × 3. In combination regimens, PF was administered 6 hours before 5ACR. The response to therapy was determined by comparing the median survival time (excluding long-term survivors) of treated groups, which consisted of eight mice each, with that of untreated control groups (24 mice). The toxicity of PF followed by 5ACR was evaluated in tumor-bearing $CD2F_1$ male mice, and was quantitated by the method described by Skipper (20).

RESULTS

Toxicity.—On the treatment regimen (every 4 days × 3, ip) used in the therapeutic studies reported herein, the LD10 levels of 5ACR and PF for male $CD2F_1$ mice were 14 and 16 mg/kg respectively. The lethal toxicity of the combination of PF followed after 6 hours by 5ACR was determined by the method described by Skipper (20), in which the sum of the decimal fraction of the LD10s for the individual drugs is plotted against the observed mortality. The actual LD10 (sum) for the combination was determined to be 0.77 from the line of best fit (fig 1). Thus, the combination of PF followed by 5ACR has greater than additive toxicity in mice.

Antitumor activity.—The combination was evaluated in mice bearing ip L1210 leukemia. In the first experiment (fig 2) treatment was initiated on Day 1. PF alone had a low order of activity (43% increase in lifespan [ILS]) at a maximally tolerated dose of 16 mg/kg. Marginal activity (29% ILS) was also seen at 4 and 8 mg/kg in this experiment. 5ACR was highly active, producing increases in lifespan of 171% and 179% at 10 or 20 mg/kg. The combination of 10 mg/kg of 5ACR with low doses of PF was superior to 5ACR alone. It is also evident that the activity of suboptimal doses of 5ACR (1.25–5 mg/kg) was clearly enhanced by pretreatment with PF. Against advanced L1210 (treatment on Days 4, 8, and 12) the results were consistent with those observed with early treatment. As shown in figure 3, PF alone had marginal activity (31% ILS) at the maximally tolerated dose, whereas 5ACR produced 62% ILS at 10 mg/kg. The combination of 5 mg/kg of 5ACR with several dose levels of PF increased median survival time by 81%–119%. Again, PF pretreatment enhanced the antitumor activity of suboptimal doses of 5ACR. For example, 2.5 mg/kg of 5ACR plus 4 mg/kg of PF, doses which had no activity (< 20% ILS) when used alone, produced 62% ILS in combination. Against early P388 leukemia (fig 4) the combination was not superior to a maximally tolerated dose of 5ACR alone (159% ILS). However, as in L1210 leukemia, the antitumor activity of low doses of 5ACR was markedly enhanced by pretreatment with PF. In this tumor system,

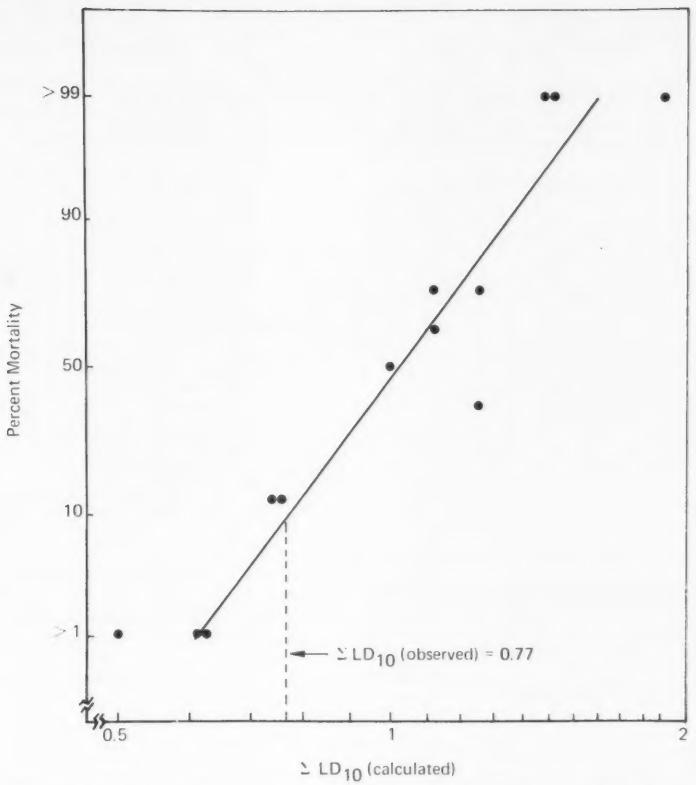

FIGURE 1.—Lethal toxicity of PF followed in 6 hrs by 5ACR to male $CD2F_1$ mice. Drugs were administered q4days × 3 to groups of 8 mice. The sum of decimal fractions of the LD10s of both agents is plotted vs % mortality. The lethality curve for the combination was plotted by regression (r = 0.93).

PF alone produced only a 23% ILS at its maximally tolerated dose. Against advanced P388 leukemia, therapeutic synergism for PF plus 5ACR was clearly evident (fig 5). PF alone was ineffective and 5ACR alone was active (55% ILS) only at 10 mg/kg. The combination of 5 mg/kg of 5ACR with 0.5–4 mg/kg of PF produced an 82%–95% ILS. Again, lower doses of 5ACR were also made more effective by PF pretreatment. Both PF alone and 5ACR alone were ineffective in mice bearing early ip colon carcinoma 26 (fig 6). The combination, on the other hand, had moderate but significant activity (> 40% ILS) at several dose levels. The greatest response was a 67% ILS at 10 mg/kg of 5ACR plus 8 mg/kg of PF. This combination dose level was toxic in other experiments. However, ILSs of 42%–47% were observed at lower doses of the two drugs as well.

DISCUSSION

The sequential combination of PF followed by 5ACR produced significant enhancement of antitumor activity against two murine leukemias relative to 5ACR alone. The combination also showed some activity against colon carcinoma 26, a solid tumor refractory to both agents when used individually. This combination has been studied extensively at the tissue culture and biochemical levels (16,17). The results of the in vivo studies are in good agreement with the in vitro findings, in that PF pretreatment appears to markedly enhance the cytotoxicity of 5ACR. It is possible that the therapeutic synergism which we have observed may be due to an enhancement of the cytotoxicity of PF by 5ACR, but this seems unlikely based on in vitro studies (16).

PF pretreatment shifts the dose-response curves of 5ACR for both toxicity and antitumor activity to lower doses of 5ACR. It is readily apparent from our studies that PF influences the host toxicity of 5ACR in a manner similar to the effect of the combination on tumor cells. The "combination toxicity index" (as defined by Skipper [20]) for PF followed by 5ACR is lower than the index

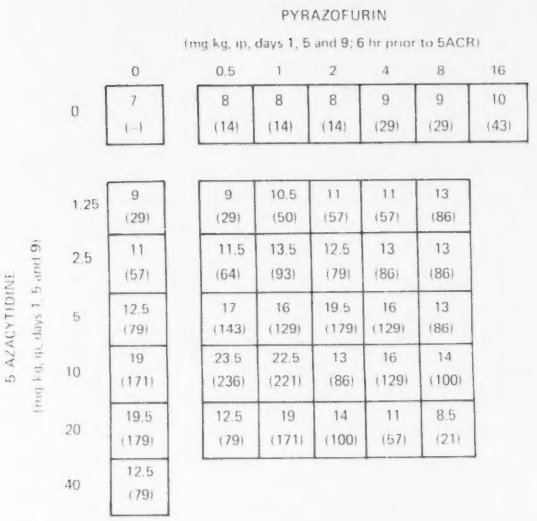

FIGURE 2.—Activity of PF followed in 6 hrs by 5ACR against early ip L1210 leukemia. 10^6 L1210 cells were inoculated ip in groups of 8 $B6D2F_1$ male mice. Nos. within the matrix are median survival time and % ILS (in parentheses). Median survival time of 24 untreated controls was 7 days as shown in the box corresponding to a 0 dose of both drugs.

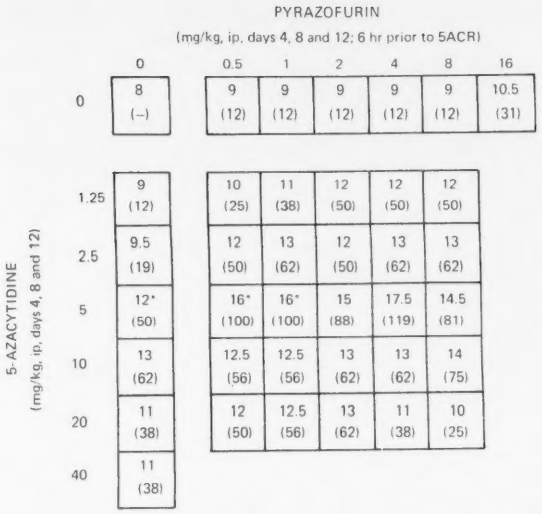

FIGURE 3.—Activity of PF followed in 6 hrs by 5ACR against advanced ip L1210 leukemia (*see* legend to fig 2). Asterisks indicate 1 tumor-free survivor among 8 mice on Day 45 (not included in calculation of median survival time).

for the majority of drug combinations reported by Skipper (20) or Schabel (21). Even though there is greater than additive toxicity to the host animal, in most experiments the combination produced greater antitumor effect at tolerated doses than was obtained with a maximally tolerated dose of 5ACR alone.

The possibility that PF pretreatment might enhance the activity of 5ACR in patients with acute myelogenous leukemia is currently under investigation.[6] Whether the combination is active in tumors refractory to both 5ACR and PF as observed in the murine colon carcinoma 26 is a question which should be explored further in experimental tumor systems. Studies suggest that a long-lasting biochemical blockade may be obtained with a fraction of the maximal tolerated clinical doses (6). Moreover, the enhanced toxicity of this combination to mice suggests that clinical trials should utilize low doses of both drugs, with cautious escalation of the 5ACR dose while maintaining the PF dose constant but sufficient to block de novo synthesis of pyrimidines.

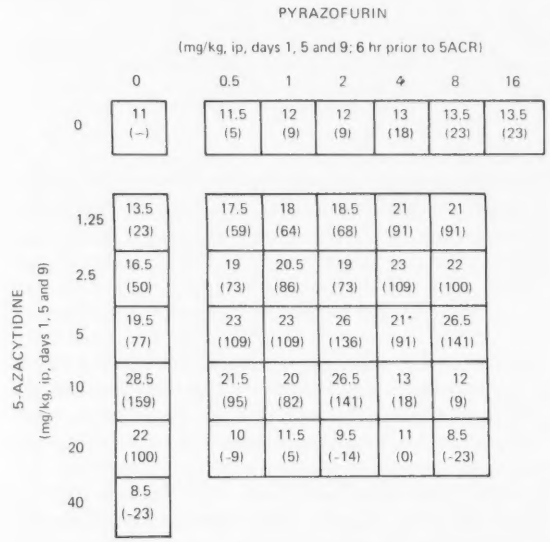

FIGURE 4.—Activity of PF followed in 6 hrs by 5ACR against early ip P388 leukemia. 10^6 P388 cells were inoculated ip in groups of 8 $B6D2F_1$ male mice (*see* legend to fig 2). Asterisk indicates 1 tumor-free survivor among 8 mice on Day 45 (not included in calculation of median survival time).

[6]Wiernik PH (Baltimore Cancer Research Program, Division of Cancer Treatment, National Cancer Institute, Baltimore, MD), Cadman EC (Yale University, New Haven, CT), and Ohnuma T (Mt. Sinai Hospital, New York, NY). Personal communications.

PYRAZOFURIN

(mg/kg, ip, days 4, 8 and 12; 6 hr prior to 5 ACR)

5-AZACYTIDINE (mg kg, ip, days 4, 8 and 12)

	0	0.5	1	2	4	8
0	11 (–)	10.5 (-5)	11 (0)	11.5 (5)	10.5 (-5)	12 (9)
1.25	12 (9)	13 (18)	14 (27)	16.5 (50)	13.5 (23)	
2.5	9.5 (-13)	15.5 (41)	16.5 (50)	17.5 (59)	20 (82)	
5	10.5 (-5)	20 (82)	20.5 (86)	21.5 (95)	20.5 (86)	
10	17 (55)	16.5 † (50)	18 † (64)	16 † (45)	15.5† (41)	
20	13.5 † (23)	13 † (18)	14 † (27)	14 † (27)	11 † (0)	
40	11 † (0)					

FIGURE 5.—Activity of PF followed in 6 hrs by 5ACR against advanced ip P388 leukemia (*see* legend to fig 2). Daggers indicate > 1 death among 8 non-tumored mice evaluated in parallel with tumor-bearing mice in this experiment.

PYRAZOFURIN

(mg/kg, ip, days 1, 5 and 9; 6 hr prior to 5 ACR)

5-AZACYTIDINE (mg/kg, ip, days 1, 5 and 9)

	0	0.5	1	2	4	8	16
0	18 (–)	20 (11)	18 (0)	19 (6)	18.5 (3)	20.5 (14)	20.5 (14)
1.25	21 (17)	20 (11)	21 (17)	23 (28)	26 (44)	20* (11)	
2.5	19 (6)	20.5 (14)	25.5 (42)	26.5 47)	24 (33)	25.5 (42)	
5	19.5 (8)	22.5 (25)	22.5 (25)	21.5 (19)	22.5 (25)	19.5 (8)	
10	19.5 (8)	20.5 (14)	21.5 (19)	24.5 (36)	12.5 (-31)	30 (67)	
20	13.5 (-25)	12.5 (-31)	13 (-28)	11 (-39)	12.5 (-31)	12 (-33)	
40	11.5 (-36)						

FIGURE 6.—Activity of PF followed in 6 hrs by 5ACR against early ip colon carcinoma 26. One-half ml of a 1% tumor brei was inoculated ip in groups of 8 $CD2F_1$ male mice (*see* legend to fig 2). Asterisk indicates 1 tumor-free survivor among 8 mice on Day 60 (not included in calculation of median survival time).

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Phase II–III Chemotherapy Studies in Advanced Gastric Cancer[1]

Charles G. Moertel* and Philip T. Lavin[2]

SUMMARY

In this study, 139 eligible and evaluable patients with advanced gastric cancer, measurable disease, and no prior chemotherapy were randomized between treatment with Adriamycin alone, 5-fluorouracil (5-FU) plus mitomycin C, and 5-FU plus methyl-CCNU. Objective responses were seen in eight of 37 patients (22%) receiving Adriamycin, in 17 of 53 (32%) receiving 5-FU plus mitomycin C, and in 12 of 49 (24%) receiving 5-FU plus methyl-CCNU. Median durations of response were 4, 3½, and 8½ months, respectively. Sixty-one patients were treated after previous failure to other chemotherapy regimens. Response rates were seen in seven of 47 patients (15%) receiving Adriamycin, in two of nine receiving 5-FU plus mitomycin C, and in one of five receiving 5-FU plus methyl-CCNU. Complete responses were observed more frequently with Adriamycin and with 5-FU plus methyl-CCNU than with 5-FU plus mitomycin C. Although 5-FU plus methyl-CCNU had a slight advantage in interval to disease progression, this was not statistically significant. Median survival for all previously untreated patients was slightly > 17 weeks, and there was no meaningful or significant survival difference between the three treatment groups.

[Cancer Treat Rep 63:1863–1869, 1979]

Although carcinoma of the stomach has shown a decreasing incidence in the US for several decades, it remains one of the leading causes of cancer death, claiming > 14,000 lives in 1978 (1). Traditional surgical treatment has proved adequate for only a small minority of patients with gastric cancer and the national end results show that almost 90% of those afflicted with the disease will die of it. Nonsurgical management of this large group of patients with advanced disease provides a major challenge for the oncologist.

Single-agent chemotherapy for gastric cancer has produced responses with a variety of drugs including the

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[2]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare. The following members participated in the study: Mayo Clinic and Mayo Foundation, Rochester, MN (*C. G. Moertel*, CA-13650), and Sidney Farber Cancer Institute, Boston, MA (*P. T. Lavin*, CA-23318). Other participating institutions include: Albany Medical College, NY (CA-06594); Albert Einstein College of Medicine, Bronx, NY (CA-14958); American Oncologic Hospital, Philadelphia, PA (CA-18281); Baystate Medical Center, Springfield, MA (CA-20182); Brookdale Hospital Center, Brooklyn, NY; Brown University, Providence, RI (CA-15947); Case Western Reserve University, Cleveland, OH (CA-14548); Chicago Medical School, IL (CA-14144); Georgetown University School of Medicine, Washington, DC (CA-02824); Hahnemann Medical College, Philadelphia, PA (CA-13611); Harbor General Hospital, Torrance, CA (CA-21091); University of Florida-Jacksonville (CA-19819); Jefferson Medical College, Philadelphia, PA (CA-14215); Johns Hopkins Oncology Center, Baltimore, MD (CA-16116); University of Manitoba, Winnipeg, Canada; Mt. Sinai Medical Center, New York, NY (CA-17152); National Cancer Institute-Veterans' Administration Medical Oncology Branch, Washington, DC; New York University Medical Center, NY (CA-16395); Northwestern University Medical Center, Chicago, IL (CA-17145); University of Ottawa, Ontario, Canada; Centre Hospitalier de Bobigny, Paris, France (CA-19273); Hospital of the University of Pennsylvania, Philadelphia (CA-15488); Quebec Program, Canada (CA-21653); University of Rochester Cancer Center, NY (CA-11083); Roswell Park Memorial Institute, Buffalo, NY (CA-12296); Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (CA-10948); University of Pretoria, South Africa (CA-21693); Swiss Group, Bern, Switzerland; SUNY-Stonybrook (Veterans' Administration Hospital), Northport, NY (CA-20161); Tufts University, Walpole, MA (CA-07190); Natalie Warren Bryant Cancer Center, Tulsa, OK; Medical College of Virginia, Richmond (CA-10572); and Wisconsin Clinical Cancer Center, Madison (CA-21076).

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fluorinated pyrimidines (5-fluorouracil [5-FU] or 5-fluorodeoxyuridine), mitomycin C, and the nitrosoureas. These responses, however, have in the main been incomplete and very transient. No evidence of any contribution to patient longevity regardless of stage of disease has been produced.

Multidrug chemotherapy studies have suggested that combinations of 5-FU and the nitrosoureas, BCNU or methyl-CCNU, may produce some increase in objective response rates (2,3). Any contribution of these combinations to patient survival, however, has been very questionable. A controlled Mayo Clinic study showed that the combination of 5-FU and BCNU produced a significant survival advantage in comparison to BCNU alone, but only a borderline advantage over 5-FU alone (2). Previous Eastern Cooperative Oncology Group (ECOG) studies had comparably shown a significant survival advantage for 5-FU and methyl-CCNU over treatment with methyl-CCNU alone (3), but no survival advantage whatsoever was demonstrated over 5-FU alone (4).

Japanese investigators (5) as well as investigators at the Sloan-Kettering Memorial Hospital (6) produced favorable response rates for gastric cancer with the combination of 5-FU, mitomycin C, and cytosine arabinoside. Earlier Mayo Clinic studies, however, had shown a similar favorable response rate in a small group of cancer patients treated with just the two-drug combination of 5-FU and mitomycin C (7). A high response rate (60%) had also been reported from Wayne State University (Detroit, MI) using mitomycin C plus a 24-hour infusion of 5-FU (8). This response rate, however, was not duplicated by a later Southwest Oncology Group gastric cancer study (19%) (9).

Adriamycin has had a broad spectrum of activity in human neoplasms that regrettably has not extended to the most common gastrointestinal neoplasm, colorectal carcinoma. Studies by Frytak et al (10) did suggest possible useful activity with this agent in advanced gastric cancer.

This ECOG study was undertaken to evaluate the two-drug combination of 5-FU and mitomycin C, as well as single-agent therapy with Adriamycin, in patients with advanced gastric cancer and measurable areas of malignant disease. It was also our purpose to compare the activity of these regimens with that of the 5-FU-methyl-CCNU combination which had produced the most favorable response rate for this type of patient in our previous studies. We employed objective tumor response, interval to tumor progression, and patient survival as the endpoints for this investigation.

METHODS

All patients selected for the study had established adenocarcinoma of gastric origin with histologic confirmation of unresectable residual, recurrent, or metastatic malignant disease. It was also required that the patient have a clearly defined area of known malignant disease to serve as an objective indicator of response to therapy. Palpable hepatomegaly was acceptable if the liver was proven to contain metastasis and if a well-defined liver edge extended at least 5 cm below the xiphoid process or costal margin on quiet respiration. A positive liver scan was acceptable if there was a clearly defined perfusion defect measuring at least 5 cm in diameter. Lesions visualized only by contrast roentgenography or lesions for which size could only be estimated, eg, pelvic or extrarectal masses, were not acceptable. Pathophysiologic changes alone, eg, jaundice, ascites, and elevated carcinoembryonic antigen, were also not considered acceptable. Contraindications to entry in the study included the following: recent surgery with resection or bypass (< 3 weeks previously), recent abdominal exploration and biopsy only (< 2 weeks previously), recent radiation therapy or chemotherapy (< 4 weeks previously), total disability (ECOG performance status of 4), severe malnutrition, severe nausea or frequent vomiting, an active infectious process, and leukopenia (< 4000 cells/mm^3) or thrombocytopenia (< 150,000 cells/mm^3). Evidence of active heart disease was a contraindication for Adriamycin therapy only.

Patients were stratified according to performance status and to whether the primary gastric tumor had been resected. Patients who had not had previous chemotherapy and who had no evidence of active heart disease were randomized between Adriamycin alone, 5-FU plus mitomycin C, and 5-FU plus methyl-CCNU. Patients with active heart disease were randomized between 5-FU plus mitomycin C and 5-FU plus methyl-CCNU. Patients with prior chemotherapy (usually 5-FU) were directly assigned to receive Adriamycin alone. Adriamycin alone was given by rapid iv push at a dose of 60 mg/m^2 on Days 1, 22, and 43 and every 4 weeks thereafter. If the patient had any elevation of direct-reacting serum bilirubin, the dose was reduced to 40 mg/m^2. Adriamycin was stopped after a total dose of 550 mg/m^2. For the 5-FU-mitomycin C combination, both drugs were given by rapid push into the tubing of an established iv infusion. 5-FU was given at a dose of 325 mg/m^2/day × 5 days on Days 1–5 and mitomycin C was given at a dose of 3.75 mg/m^2/day × 5 days iv on Days 1–5. This regimen corresponded closely to that of the earlier Mayo Clinic study (7). Courses of therapy were repeated every 5 weeks. For the 5-FU-methyl-CCNU combination, methyl-CCNU was given orally in a single dose of 150 mg/m^2 on Day 1 and 5-FU was given by rapid iv injection at a dose of 325 mg/m^2/day × 5 days on Days 1–5 and 375 mg/m^2/day × 5 days on Days 36–40. Cycles of therapy were repeated every 10 weeks.

For patients who had disease progression with their

TABLE 1.—Characteristics of 115 patients with no prior chemotherapy and no heart disease

Characteristic	Treatment		
	Adriamycin	5-FU + mitomycin C	5-FU + methyl-CCNU
Total No. of patients	37	41	37
Sex (M/F ratio)	2.1	1.7	1.8
Age in yrs			
Median	58	62	62
Range	47–81	42–72	27–79
% of patients with—			
Primary tumor resected	68	51	76
Degree of differentiation			
Well	27	10	14
Moderate	24	51	41
Poor	46	34	45
Unknown	3	5	—
Liver involvement	62	63	54
Initial ECOG performance status			
0–1	59	55	62
2–3	41	45	38
Weight loss in prior 6 mos			
None	11	22	3
< 5%	11	24	27
5%–10%	35	17	27
> 10%	41	37	38
Unknown	3	—	5

original randomized assigned regimen and who remained eligible for treatment, those who were initially randomized to Adriamycin alone were randomized for crossover treatment between 5-FU plus mitomycin C and 5-FU plus methyl-CCNU and those who were initially randomized to either of the two drug combinations and who had no heart disease were assigned to treatment with Adriamycin alone.

It was recommended that leukocyte and platelet counts be taken for each regimen twice weekly after the first course and once weekly thereafter. Appropriate reductions in doses of subsequent courses were made if excessive toxicity was experienced.

Patients were re-evaluated before each dose of Adriamycin and before each course of combination chemotherapy. For those patients with measurable disease, an objective response was declared if all of the following criteria were met: (*a*) a reduction by at least 50% of the product of the longest perpendicular diameters of the most clearly measurable tumor lesion (if hepatomegaly was the indicator lesion it was required that there be a reduction of the sum of measurements below the xiphoid and each costal margin by at least 30%); (*b*) no increase in the size of any other measurable lesions and no new lesions of malignant disease; (*c*) no significant loss in body weight (> 5%) or deterioration of performance status (one level by ECOG score); and (*d*) continuation of the response for at least 6 weeks after the start of therapy. Disease progression was declared if any of the following criteria were met: (*a*) increase in size of any measurable lesion by > 25%; (*b*) appearance of new areas of malignant disease; or (*c*) loss of weight (> 5%) or deterioration in performance status (one level by ECOG score).

RESULTS

A total of 191 patients were entered in the study and started treatment at 37 participating institutions. Thirty-two patients had had prior chemotherapy and were assigned directly to receive Adriamycin alone. One hundred and thirty-one patients were randomized to the three treatment arms. Twenty-eight patients with heart disease were randomized between the two 5-FU-containing treatment arms. Twenty-five patients were declared ineligible or unevaluable, primarily because of failure to meet protocol entry criteria or because of gross failure in administering protocol treatment regimens. One hundred and sixty-six patients were therefore fully eligible and evaluable for both objective response and survival analysis. All eligible patients who were properly initiated in therapy were included in this analysis. Specifically, patients were not declared unevaluable if they died soon after treatment or if they became too sick to continue treatment.

The pretreatment characteristics of our randomized patient group are shown in table 1. The treatment groups

TABLE 2.—Toxicity with initial course of therapy

Toxic reactions	No. of patients with toxic reactions/total No. treated		
	Adriamycin	5-FU + mitomycin C	5-FU + methyl-CCNU
Nausea and vomiting	50/84 (60%)	32/62 (52%)	35/56 (63%)
Diarrhea	1/84 (1%)	11/62 (18%)	4/56 (7%)
Stomatitis	2/84 (2%)	2/62 (3%)	2/56 (4%)
Leukopenia (cells/mm^3)			
< 4000 ≥ 2000	23/51 (45%)	17/32 (53%)	16/34 (47%)
< 2000	15/51 (29%)	6/32 (19%)	9/34 (26%)
Thrombocytopenia (cells/mm^3)			
< 150,000 ≥ 50,000	12/48 (25%)	12/30 (40%)	22/32 (69%)
< 50,000	5/48 (11%)	9/30 (30%)	6/32 (19%)

TABLE 3.—Hematologic toxicity of Adriamycin according to prior chemotherapy exposure

Toxic reactions	No. of patients with toxic reactions/total No. treated	
	Prior chemotherapy	No prior chemotherapy
Leukopenia (cells/mm^3)		
< 4000 ≥ 2000	18/30 (60%)	5/21 (25%)
< 2000	7/30 (23%)	8/21 (38%)
Thrombocytopenia (cells/mm^3)		
< 150,000 ≥ 50,000	7/27 (26%)	5/21 (24%)
< 50,000	4/27 (15%)	1/21 (5%)

were reasonably balanced with respect to prognostic determinants.

Toxicity.—Toxic reactions experienced by all patients in this study, with both initial and crossover treatment, are shown in table 2 for those patients in whom toxicity was adequately documented. Nausea and vomiting was a frequent reaction to all regimens. Mucocutaneous reactions, as well as diarrhea, were infrequent and mild. Leukopenia was experienced by the majority of patients in each treatment arm and with essentially the same frequency and severity. Thrombocytopenia was less frequent in the Adriamycin-treated patients. Table 3 shows the hematologic toxicity of Adriamycin according to prior chemotherapy exposure. Toxic reactions were more frequent in the previously treated group. Numbers are too small in prior chemotherapy groups for any meaningful analysis of the other treatment arms. Increasing hematologic toxicity with repeated courses of treatment was clearly demonstrated by 12 patients receiving 5-FU plus mitomycin C and by three patients receiving 5-FU plus methyl-CCNU. The true frequency of such compounding of hematologic toxicity is difficult to estimate because of dose adjustments that were made during the course of therapy, but this clearly became a major problem in a number of patients receiving prolonged treatment with 5-FU plus mitomycin C. There was one treatment-related death among those treated with 5-FU plus mitomycin C and one death among those treated with 5-FU plus methyl-CCNU. In each case death was caused by severe leukopenia and complicating sepsis.

Objective response to therapy.—The objective responses to treatment among patients with measurable disease are recorded in table 4. Activity was seen with all regimens, and the differences in response rates, for both initial and crossover therapy, were not statistically significant. Median durations of response among previously untreated patients measured from the day of initiation of therapy were 4 months (range, 1.5–12.5+) for Adriamycin, 3½ months (range, 2.5–12) for 5-FU plus mitomycin C, and 8½ months (range, 3–12.5) for 5-FU

TABLE 4.—Objective response to therapy

Regimen	No. of patients responding/total No. treated		
	No prior chemotherapy	Prior chemotherapy	Totals
Adriamycin	8/37 (22%)	7/47 (15%)	15/85 (18%)
5-FU + mitomycin C	17/53 (32%)	2/9	19/62 (31%)
5-FU + methyl-CCNU	12/49 (24%)	1/5	13/54 (24%)

TABLE 5.—Objective response to therapy according to site of measurable disease

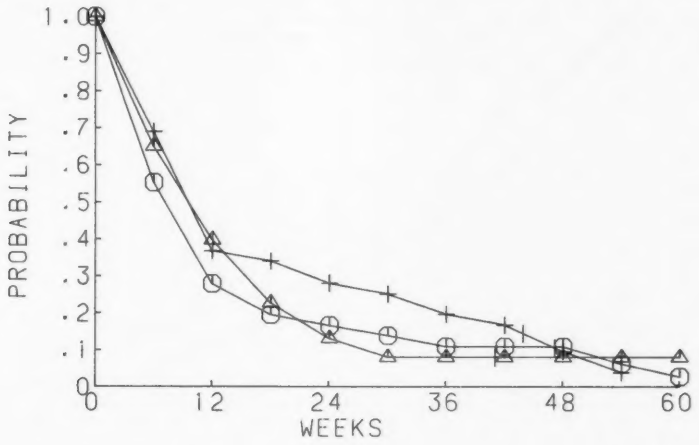
Indicator lesion*	No. of patients responding/total No. treated			
	Adriamycin	5-FU + mitomycin C	5-FU + methyl-CCNU	Totals
Liver	3/35	11/31	6/24	20/90 (22%)
Abdominal	6/29	5/22	2/18	13/69 (19%)
Nodal	2/13	3/11	3/11	8/35 (23%)
Pulmonary	2/7	0/7	1/8	3/22 (14%)
Subcutaneous	2/9	3/6	0/2	5/17 (29%)
Osseous	2/4	—	1/2	3/6 (50%)

*Total No. of sites of indicator lesions exceeds total No. of patients because several patients had multiple sites.

plus methyl-CCNU. With respect to duration of response, 5-FU plus methyl-CCNU was significantly superior to 5-FU plus mitomycin C ($P < 0.01$, Gehan-Wilcoxon; $P < 0.02$, log-rank) but not to Adriamycin. In prior chemotherapy or crossover-treated patients, median duration of response for Adriamycin was 2 months (range, 1.5–38+). Among those patients with no prior chemotherapy, complete responses were observed in five of 18 who responded to Adriamycin, in three of 17 who responded to 5-FU plus mitomycin C, and in nine of 12 ($P < 0.01$) who responded to 5-FU plus methyl-CCNU. Objective response to therapy in relation to sites of measurable disease is shown in table 5.

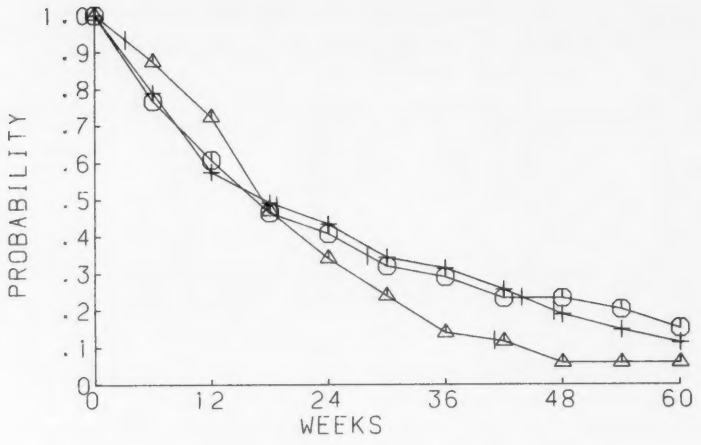
Disease progression.—Times to disease progression measured from the beginning of therapy for patients with no prior chemotherapy and no active heart disease are shown in figure 1. There was no significant difference between the three treatment arms. Including patients with active heart disease, the median times to progression were 10 weeks for 53 patients initially treated with 5-FU plus mitomycin C and 8 weeks for 49 patients initially treated with 5-FU plus methyl-CCNU, again with no significant difference between these two treatment arms. Median times to disease progression for patients with prior chemotherapy were 7 weeks for Adriamycin, 5 weeks for 5-FU plus mitomycin C, and 10 weeks for 5-FU plus methyl-CCNU.

Survival.—Survival times from the beginning of treat-



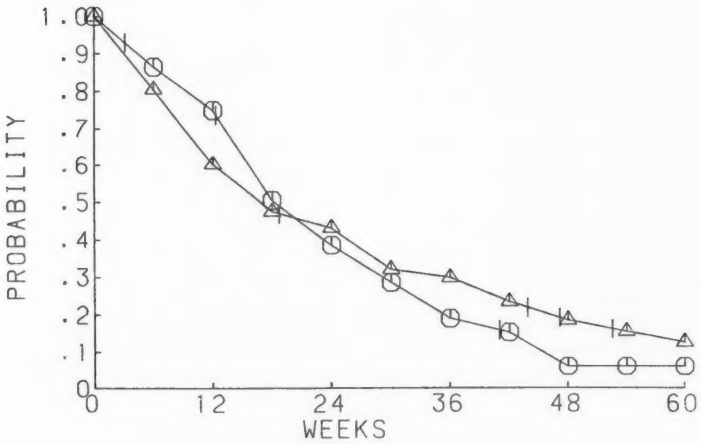
TREATMENT CODE	NO PROG	PROG	TOTAL	MEDIAN
⊖ ADRIAMYCIN	0	37	37	6.9
△ 5 - FU + MITOMYCIN C	2	39	41	9.2
+ 5 - FU + METHYL-CCNU	2	35	37	9.1

FIGURE 1.—Time to disease progression among 115 patients with no prior chemotherapy and no heart disease.



TREATMENT	CODE	ALIVE	DEAD	TOTAL	MEDIAN
ADRIAMYCIN	2	2	35	37	16.3
5 - FU + MITOMYCIN C	3	3	38	41	17.1
5 - FU + METHYL-CCNU	4	4	33	37	17.4

FIGURE 2.—Survival among 115 patients with prior chemotherapy and no heart disease.



TREATMENT	ALIVE	DEAD	TOTAL	MEDIAN
5-FU + Mitomycin C	4	49	53	18.2
5-FU + Methyl-CCNU	5	44	49	16.7

FIGURE 3.—Survival among 102 patients with no prior chemotherapy (patients with heart disease included).

ment among the 115 patients with no prior chemotherapy and no heart disease are shown in figure 2. The survival curves essentially overlap with a median survival of 17 weeks for the entire group. Cox analysis does not change these relationships. In figure 3 survival time is shown for all patients randomized to the non-Adriamycin treatment arms. These include 24 patients with heart disease who are not included in figure 2. Again, there is no significant difference with a median survival of slightly > 17 weeks. Median survival in patients with prior chemotherapy was 19 weeks for those treated with Adriamycin alone, 14 weeks for those treated with 5-FU plus methyl-CCNU, and only 5 weeks for those treated with 5-FU plus mitomycin C.

DISCUSSION

This study has demonstrated the activity of Adriamycin in a large group of patients with advanced gastric carcinoma. The objective response rates observed with this agent were 22% in patients without prior chemotherapy and 15% in those with previous cytotoxic drug exposure. Although response rates with the two-drug combinations were somewhat higher, this advantage could not be demonstrated with statistical significance. It is interesting to note that the 24% response rate with combined 5-FU and methyl-CCNU is considerably less than the 40% response rate recorded with the same combination in an earlier ECOG study employing essentially the same methodology. The three regimens were essentially identical with regard to interval to disease progression and patient survival.

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Phase II–III Chemotherapy Studies in Advanced Gastric Cancer[1]

The Gastrointestinal Tumor Study Group[2,*]

SUMMARY

In this multi-institutional study of advanced gastric cancer, 141 eligible and evaluable patients were treated with either (*a*) Adriamycin alone, (*b*) a combination of 5-fluorouracil, Adriamycin, and methyl-CCNU (FAMe), or (*c*) a combination of 5-fluorouracil, mitomycin C, and cytosine arabinoside (FMC). In patients with measurable disease and no prior chemotherapy, objective responses were seen in four of 17 patients (24%) receiving Adriamycin alone, in seven of 15 (47%) receiving FAMe, and in three of 18 (17%) receiving FMC. In previously treated patients with measurable disease, objective responses were seen in three of 17 patients (18%) receiving Adriamycin alone and in one of 11 (9%) receiving FMC. In previously untreated patients with both measurable and nonmeasurable disease, FAMe showed a significant advantage over both Adriamycin alone and FMC with regard to interval to disease progression and survival. In comparing Adriamycin alone with FMC in previously treated patients, intervals to disease progression and survival were essentially identical.

[Cancer Treat Rep 63:1871–1876, 1979]

Although advanced gastric carcinoma has shown responsiveness to several cytotoxic drugs, most notably 5-fluorouracil (5-FU) and mitomycin C, response rates have only been in the 15%–30% range with no evidence of a significant contribution to patient survival. Particularly disappointing has been the failure of single drugs as adjuvants to potentially curable gastric surgery.

There have been suggestions in the literature that drug combinations involving 5-FU and either mitomycin C or the nitrosoureas may increase objective response rates (1–3). There has also been evidence in earlier studies that the combination of 5-FU and either BCNU or methyl-CCNU may contribute to the length of survival in advanced gastric carcinoma when compared to single-drug treatment (2,3). A more recent Eastern Cooperative Oncology Group (ECOG) study, however, has failed to show a survival advantage of treatment with combined 5-FU and methyl-CCNU when compared to treatment with 5-FU alone (4).

There still exists a strong need to seek out new active single agents for therapy for gastric cancer and there would also seem to be ample justification for further exploration of drug combinations. The purposes of this Gastrointestinal Tumor Study Group investigation included first an evaluation of single-drug therapy with Adriamycin, an agent which showed activity for gastric carcinoma in early Mayo Clinic studies (5). Second, it was postulated that perhaps Adriamycin could contribute to the activity of 5-FU and methyl-CCNU in a three-drug combination. And third, we wanted to evaluate the combination of 5-FU, mitomycin C, and cytosine arabinoside which has been reported to have substantial therapeutic activity in Japanese studies (6) and in a study conducted at the Sloan-Kettering Memorial Hospital (New York, NY) (7). For this purpose, we elected to employ an intensive course schedule rather than the intermittent schedule used in the earlier studies.

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[2]This study was conducted by the Gastrointestinal Tumor Study Group. The following institutions (with investigators) participated in the study: Frontier Science and Technology Research Foundation Inc, Boston, MA (*P. T. Lavin*); Mayo Clinic, Rochester, MN (*C. G. Moertel, E. T. Creagan, S. Frytak, R. G. Hahn, M. J. O'Connell, R. J. Reitemeier, J. Rubin,* and *A. J. Schutt*); Sidney Farber Cancer Institute, Boston, MA (*J. Lokich, J. Brooks,* and *J. Corson*); Mt. Sinai School of Medicine, New York, NY (*H. Bruckner* and *B. Kabakow*); Roswell Park Memorial Institute, Buffalo, NY (*H. Douglass, Jr, H. Nava,* and *F. Nime*); Yale University School of Medicine, New Haven, CT (*E. Livstone, H. Spiro,* and *J. Marsh*).

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METHODS

All patients selected for study were required to have known primary gastric adenocarcinoma with histologic proof of unresectable residual, recurrent, or metastatic disease. Patients with both measurable and nonmeasurable disease were accepted but were separately stratified. If a patient was accepted as having measurable disease, it was required that there be an area of known tumor which could be clearly calibrated. Palpable hepatomegaly was acceptable if the liver was proven to contain metastasis and if a well-defined liver edge extended at least 5 cm below the xiphoid process or costal margin on quiet respiration. A positive liver scan was accepted if there was a clearly defined perfusion defect measuring at least 5 cm in diameter. Lesions visualized only by contrast roentgenography or lesions for which size could only be estimated, eg, pelvic or extrarectal masses, were not accepted. Contraindications to entry in the study included the following: recent surgery with resection or bypass (< 3 weeks previously); recent abdominal exploration and biopsy only (< 2 weeks previously); recent radiation therapy or chemotherapy (< 4 weeks previously); total disability (ECOG performance score of 4); severe malnutrition or severe nausea or frequent vomiting; an active infectious process; and leukopenia (< 4000 cells/mm^3) or thrombocytopenia (< 150,000 cells/mm^3). Evidence of active heart disease was a contraindication for entry in either of the two Adriamycin-containing treatment arms.

Patients were stratified according to whether they had measurable or nonmeasurable disease, according to performance status, and according to whether the primary gastric tumor had been resected. Patients who had not had previous chemotherapy with any of the agents under study and who had no evidence of active heart disease were randomized between treatment with Adriamycin alone, 5-FU, Adriamycin, and methyl-CCNU (FAMe), and 5-FU, mitomycin C, and cytosine arabinoside (FMC). Patients with active heart disease were assigned to FMC treatment. Patients who were previously treated with any of the constituent drugs of the combinations except Adriamycin were assigned to Adriamycin alone. Patients who showed treatment failure after the FAMe combination were dropped from the study; patients who showed failure after the FMC combination were assigned to Adriamycin alone; and patients who showed failure after Adriamycin alone were assigned to the FMC combination.

Adriamycin alone was given by rapid iv push at a dose of 60 mg/m^2 on Days 1, 22, and 43 and every 4 weeks thereafter. If the patient had any elevation of direct-reacting serum bilirubin, the dose was reduced to 40 mg/m^2. Adriamycin was stopped after a total dose of 550 mg/m^2 was given. Dose regimens for the combinations were determined by pilot study as those producing definite but clinically tolerable hematologic toxicity. For the FMC combination, all drugs were given by rapid push into the tubing of an established iv infusion. The following dose regimens were employed: 5-FU, 300 mg/m^2/day × 5 on Days 1–5; mitomycin C, 1 mg/m^2/day × 5 on Days 1–5; and cytosine arabinoside, 50 mg/m^2/day × 5 on Days 1–5. These courses were repeated every 5 weeks. For the FAMe combination, the following dose regimens were employed: 5-FU, 350 mg/m^2/day × 5 by rapid iv push on Days 1–5 and 36–40; Adriamycin, 40 mg/m^2 by rapid iv push on Days 1 and 36; and methyl-CCNU, 150 mg/m^2 orally on Day 1. This cycle was repeated every 10 weeks. Adriamycin was discontinued after a total dose of 550 mg/m^2 was given and treatment was continued with 5-FU and methyl-CCNU. Treatment with any of the three regimens was discontinued with evidence of disease progression (vide infra).

For each regimen, leukocyte and platelet counts were recommended twice weekly after the first course and once weekly thereafter. Appropriate reductions in doses of subsequent courses were made if excessive toxicity was observed.

Patients were re-evaluated before each dose of Adriamycin and before each course of combination therapy. For those patients with measurable disease, an objective response was achieved if all of the following criteria were met: (*a*) a reduction by at least 50% of the product of the longest perpendicular diameter of the most clearly measurable tumor lesion (if hepatomegaly was the indicator lesion, it was required that there be a reduction of the sum of measurements below the xiphoid and each costal margin by at least 30%); (*b*) no increase in the size of any other measurable lesions and no new lesions of malignant disease; (*c*) no significant loss in body weight (> 5%) or deterioration of performance status (one level by ECOG score); and (*d*) continuation of this result for at least 6 weeks after the start of therapy. This means that a patient must demonstrate a response at Week 6 of treatment or later. No minimum duration of this response was required. For patients with either measurable or nonmeasurable disease, disease progression was achieved if any of the following criteria were met: (*a*) an increase in size of any measurable lesion by > 25%; (*b*) the appearance of new areas of malignant disease; and (*c*) a loss in body weight (> 5%) or a deterioration in performance status (one level by ECOG score).

RESULTS

A total of 174 patients were entered in the study by six participating institutions. Nine of these patient entries were cancelled before treatment was administered. Twenty-four patients had had prior chemotherapy with one of the agents in the combinations other than Adriamycin and were directly assigned to receive Adriamycin alone. Seven patients had active heart disease and were

TABLE 1.—Characteristics of randomized patients with no prior chemotherapy and no heart disease

Characteristic	Treatment		
	Adriamycin	FMC	FAMe
Total No. of patients	36	36	38
Sex (M/F ratio)	2.6	1.6	2.5
Age in yrs			
Median	56	59	63
Range	28–79	35–81	31–80
% of patients with—			
Measurable disease	47	39	39
Primary tumor resected	64	44	55
Degree of differentiation			
Well	17	11	11
Moderate	33	33	45
Poor	47	42	42
Unknown	3	14	2
Liver involvement	58	42	39
Initial ECOG performance status			
0–1	47	64	63
2–3	53	36	37
Weight loss in prior 6 mos			
None	19	11	16
< 5%	11	36	18
5%–10%	28	11	29
> 10%	36	36	32
Unknown	6	6	5

directly assigned to receive FMC. One hundred and thirty-four patients were randomized to the three treatment arms. Twenty-four of these patients were declared ineligible, 15 because of failure to meet protocol entry criteria and nine because of gross error in administering protocol dose or regimen. The remaining 110 patients were evaluated in our randomized comparison. All eligible patients who received proper initial therapy were included in this analysis. Specifically, patients were still considered to be evaluable even if they died soon after initiating treatment or if they became too sick to continue treatment.

The pretreatment characteristics of our randomized patient group are shown in table 1.

Toxicity.—Toxic reactions experienced by all patients in this study with both initial and crossover treatment are shown in table 2 for those patients with adequate documentation of toxicity. Toxicity was recorded for the first course of therapy only. Nausea and vomiting was the most frequent toxic effect in the combination-treated

TABLE 2.—Toxicity with initial course of therapy only

Toxic reactions	No. of patients with toxic reactions/total No. treated		
	Adriamycin	FMC	FAMe
Nausea and vomiting	18/72 (25%)	32/59 (54%)	27/38 (71%)
Diarrhea	1/72 (1%)	2/59 (3%)	0/38
Stomatitis	6/72 (8%)	5/59 (8%)	6/38 (16%)
Leukopenia (cells/mm^3)			
< 4000 > 2000	21/56 (38%)	19/48 (40%)	13/30 (43%)
< 2000	20/56 (36%)	18/48 (37%)	14/30 (47%)
Thrombocytopenia (cells/mm^3)			
< 150,000 > 50,000	9/47 (19%)	18/46 (39%)	17/29 (59%)
< 50,000	1/47 (2%)	9/46 (20%)	6/29 (21%)

TABLE 3.—Hematologic toxicity and prior chemotherapy

Toxic reactions	No. of patients with toxic reactions/total No. treated			
	Adriamycin		FMC	
	Prior chemotherapy	No prior chemotherapy	Prior chemotherapy	No prior chemotherapy
Leukopenia (cells/mm^3)				
< 4000 > 2000	5/24 (21%)	16/32 (50%)	8/13 (62%)	11/35 (31%)
< 2000	13/24 (54%)	7/32 (22%)	3/13 (23%)	15/35 (43%)
Thrombocytopenia (cells/mm^3)				
< 150,000 > 50,000	4/21 (19%)	5/26 (19%)	7/13 (54%)	11/33 (33%)
< 50,000	1/21 (5%)	0/26	1/13 (8%)	8/33 (24%)

patients; for the FAMe regimen, however, this was primarily limited to the day of methyl-CCNU administration. Mucocutaneous toxicity was infrequent and mild. Leukopenia was demonstrated by the majority of patients treated in each regimen and was somewhat more prominent with FAMe. Thrombocytopenia was both more frequent and more severe with the combination regimens. Compounding of hematologic toxicity with repeated treatment was particularly evident with the FMC regimen. This was documented in patients both with and without prior chemotherapy. Table 3 shows hematologic toxicity in relation to previous chemotherapy. It is not surprising that Adriamycin shows increased reactions in previously treated patients since such patients were crossed over from regimens containing either mitomycin C or nitrosoureas, both of which are known to produce cumulative marrow toxicity. The more severe toxicity with FMC in previously untreated patients defies explanation and is perhaps an artifact. One patient showed evidence of cardiotoxicity probably related to Adriamycin, but only four patients were treated to the maximum allowable Adriamycin dose of 550 mg/m^2. Among 149 total treatment experiences, there were three drug-related deaths (2%) resulting from severe marrow depression: two with FMC and one with FAMe.

Objective response to therapy.—The objective response rates to treatment among patients with measurable disease are recorded in table 4. Activity was seen with all regimens, and the response rate with the FAMe regimen in untreated patients seemed particularly favorable. The numbers of patients are relatively small, however, and the differences in response rate are not statistically significant. Complete responses were recorded for three patients treated with Adriamycin alone, for three treated with FAMe, and for one treated with FMC. Table 5 shows the response rates according to indicator sites. None of these differences are statistically significant. Respective median durations of response among previously un-

TABLE 4.—Objective response to therapy

Regimen	No. of patients responding/total No. treated		
	No prior chemotherapy	Prior chemotherapy	Totals
Adriamycin	4/17 (24%)	3/17 (18%)	7/34 (21%)
FMC	3/18 (17%)	1/11 (9%)	4/29 (14%)
FAMe	7/15 (47%)	—	7/15 (47%)

TABLE 5.—Objective response according to site of measurable disease

Measurable indicator lesions*	No. of patients responding/total No. treated			
	Adriamycin	FMC	FAMe	Totals
Liver	4/20	1/14	2/8	7/42 (17%)
Abdominal	1/5	2/5	1/3	4/13 (31%)
Subcutaneous	1/6	0/4	1/2	2/12 (17%)
Nodal	1/8	1/6	3/3	5/17 (29%)
Pulmonary	1/4	0/3	—	1/7 (14%)
Osseous	0/1	0/1	—	0/2

*Total No. of sites exceeds total No. of patients because some patients had multiple sites.

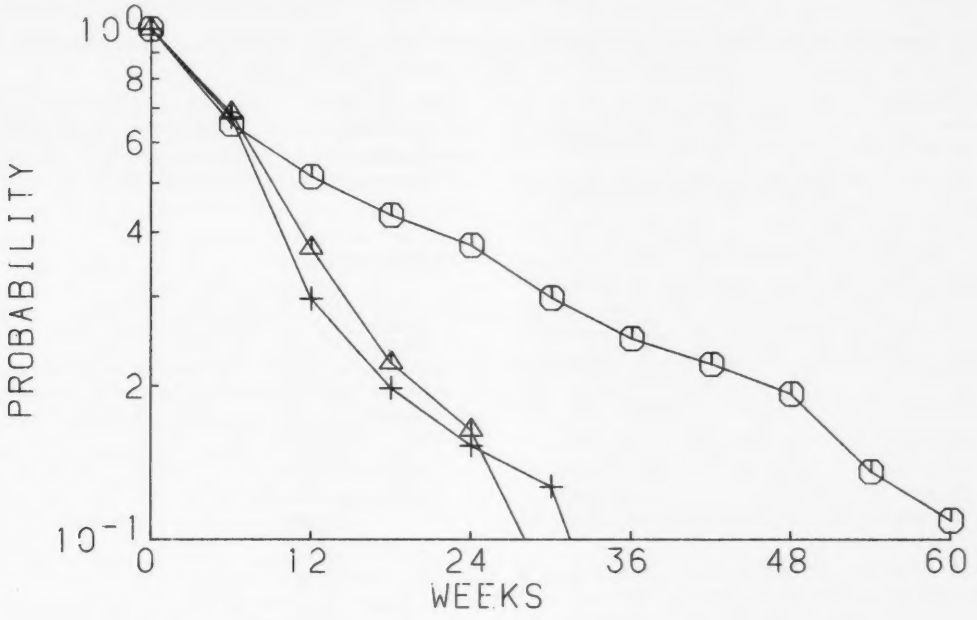
treated patients measured from the day of initiation of therapy were 6 months (range, 2.3–7.5) for Adriamycin alone, 2.5 months (range, 1.5–6) for FMC, and 7.5 months (range, 1.5–10) for FAMe. These differences are not statistically significant. The median duration of response in patients with prior chemotherapy (both those treated before entry in this study and those crossed over to secondary treatment during this study) was 3.5 months for both Adriamycin alone and FMC.

Time to progression.—Times to progression measured from the start of therapy for patients with either measurable or nonmeasurable disease with no prior chemotherapy and no active heart disease are shown in figure 1. FAMe was superior to both Adriamycin alone and FMC at $P < 0.03$ and $P < 0.04$ levels of statistical significance. Although time to disease progression is frequently not a clearly defined endpoint that may be in some measure a subjective judgment, these differences correspond closely to those of the more absolute endpoint of patient survival (vide infra). There was no difference among previously treated patients with respect to the time to disease progression: the median was only 6 weeks for both Adriamycin and FMC.

Survival.—Survival times from the start of therapy are shown in figure 2, and again FAMe has an advantage. This is particularly striking with respect to long-term survival. Using the Cox multi-variant analysis to account for variations in prognostic factors, FAMe was superior to both FMC and Adriamycin alone at a $P < 0.04$ level. There was no substantial difference with respect to survival among patients receiving secondary treatment with either Adriamycin alone or FMC: the median survival times were 13.6 and 13.0 weeks, respectively.

DISCUSSION

This study has confirmed the activity of Adriamycin for gastric carcinoma in the hands of a multi-institutional



TREATMENT	NO PROG	PROG	TOTAL	MEDIAN
○ FAMe	2	36	38	12.9
△ FMC	0	36	36	9.1
+ Adriamycin	0	36	36	8.2

FIGURE 1.—Time to disease progression by initial treatment program in randomized patients with no prior chemotherapy and no heart disease. FAMe is superior to Adriamycin alone ($P < 0.03$) and to FMC ($P < 0.04$).

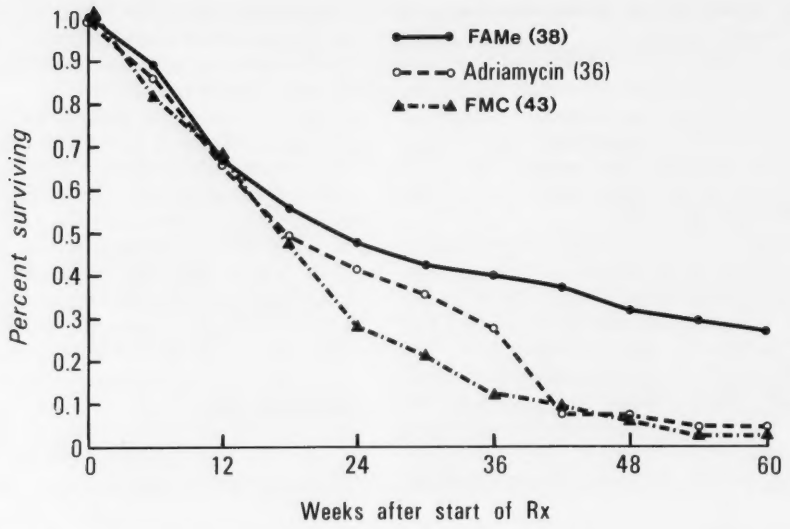


FIGURE 2.—Survival by initial treatment program in randomized patients with no prior chemotherapy and no heart disease. FAMe is superior to both Adriamycin alone and FMC ($P < 0.04$).

cooperative group. Response rates for this agent are within the range of those previously reported with 5-FU and mitomycin C. Adriamycin also has activity in patients showing disease progression following treatment with 5-FU or mitomycin C. When 5-FU and methyl-CCNU are added to Adriamycin, this three-drug combination shows therapeutic advantage when compared to Adriamycin alone or to the FMC combination. This study did not address the question of whether the same therapeutic advantage could have been obtained with the combination of 5-FU plus Adriamycin or of methyl-CCNU plus Adriamycin. It would seem appropriate to answer this question before moving this three-drug combination to surgical adjuvant trials. The preceding ECOG study (Moertel and Lavin, this issue) indicates that the two-drug combination of 5-FU and methyl-CCNU does not have any advantage over Adriamycin alone.

The FMC combination, as employed in this study, was quite disappointing. In spite of the production of significant toxicity, response rates were low, and both interval to disease progression and survival were significantly inferior to the FAMe combination. It should be emphasized, however, that the dose schedule employed in this study was not the same as that used in the Japanese and the Sloan-Kettering Memorial Hospital studies, and this difference could have had a deleterious effect on therapeutic activity.

The current Gastrointestinal Tumor Study Group investigation, now nearing completion, involves a comparison of FAMe with 5-FU plus methyl-CCNU and 5-FU plus Adriamycin plus mitomycin C, a combination recently reported to produce a high rate of response in gastric cancer by investigators at Georgetown University (8).

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Quantitative Experimental Evaluation of Adriamycin Cardiotoxicity in the Mouse[1]

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SUMMARY

Adriamycin cardiotoxicity was morphologically evaluated in mice treated iv ten times at three dose levels which were equal to fixed fractions of the LD50. Good correlation between the degenerative heart lesions, scored according to a nonparametric scoring system, and the doses was found and allowed calculation of a median cumulative cardiotoxic dose of 36.4 mg/kg of body weight (121.5 mg/m^2). The method is suitable for quantitative screening of the potential cardiotoxicity of new anthracycline analogs under standardized conditions.

[Cancer Treat Rep 63:1877–1883, 1979]

Cardiotoxicity is a major limiting complication of anthracycline antitumor agents given beyond certain cumulative doses, and is well-known both in humans and in animals (1). Improvement of cancer therapy with this class of agents is chiefly directed at new analogs, characterized by a more favorable ratio between antitumor activity and cardiotoxic effects. Thus, evaluation of the cardiotoxic effects by use of a reproducible method suitable for a screening test could be very useful. The animal generally used for experimental evaluation of anthracycline cardiotoxicity is the rabbit (2,3); however, rabbits are expensive to use and require large amounts of drugs, which may often be unavailable. Several authors have recently described morphologic, biochemical, and functional cardiac alterations induced by anthracyclines in rats (4–6) and mice (7) and have proposed procedures for testing the cardiotoxicity of new analogs.

This paper describes an in vivo method performed in the mouse essentially for this purpose. The method addresses the problems of a screening test in that it takes into account the requirements for a rational approach to the study of chronic toxic effects, such as standardized conditions of accumulation, quantitative evaluation of the specific effect on the target tissue, and statistical determination of the drug toxicity as a median cumulative cardiotoxic dose.

MATERIALS AND METHODS

Sixty CD_1 female white mice (the sex used in our experimental antitumor trials) of an average weight of 20 ± 2 g obtained from Charles River Italia (Calco-Como) were randomly distributed into groups of five, maintained in plastic cages in a standardized environment (45%–60% humidity, 21.5°C–22.5°C), and fed a laboratory diet and water ad libitum for 1 week before and during the test.

Adriamycin, dissolved in saline, was injected iv (10 ml/kg of body weight) twice a week (Monday and Thursday) ten times at doses of 1, 2, and 4 mg/kg/day (15 mice/group). One group, injected with saline alone, was utilized as a control. These doses correspond to one fifth, one tenth, and one twentieth of the single-dose LD50 as determined in female CD_1 mice 28 days after the treatment. Animals were not treated for 2 weeks between the first four injections and the last six injections to allow the recovery of bone marrow depression; 4 weeks after the last injection (ie, 11 weeks after the experiment was initiated) all mice were killed by ether asphyxiation.

The whole heart, liver, spleen, and kidneys were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.3 and then dehydrated and embedded in hydroethyl methacrylate (8). Heart frontal sections and median sections of other tissues (1 μm) were obtained with a Sorvall JB 4 A microtome, stained with 0.1% toluidine blue in 1% borax, and observed by light microscopy. The heart

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TABLE 1.—Morphologic evaluation of cardiac lesions

Degree	Definition
	Severity
1	Sarcoplasmic microvacuolizations and/or inclusions and interstitial or cellular edema
2	Same as 1 plus sarcoplasmic macrovacuolizations or atrophia, necrosis, fibrosis, endocardial lesions, and thrombi
	Extension
0	No lesions
0.5	< 10 single altered myocytes in the whole heart section
1	Scattered single altered myocytes
2	Scattered small groups of altered myocytes
3	Widely spread small groups of altered myocytes
4	Confluent groups of altered myocytes
5	Most cells damaged

lesions were evaluated on one median section and scored according to severity and extension, as shown in table 1. The product of the severity and the extent of the damage observed in each mouse gives the single scores (from 0 to 10) used for the statistical evaluation of the regression line according to the nonparametric method of Sen (9) and for the calculation of the median cumulative cardiotoxic dose (MeCCD), ie, the total dose giving a score of 5.

RESULTS

Typical heart histologic patterns of control and treated mice, showing different degrees of degenerative lesions, are reported in figures 1–6. Both severity and extension of the lesions are dose-related. At the lowest dose the microscopic lesions were represented by characteristic microvacuolization of fibers. This injury, scored as severity 1, usually involved few myocytes, with 0.5 or 1

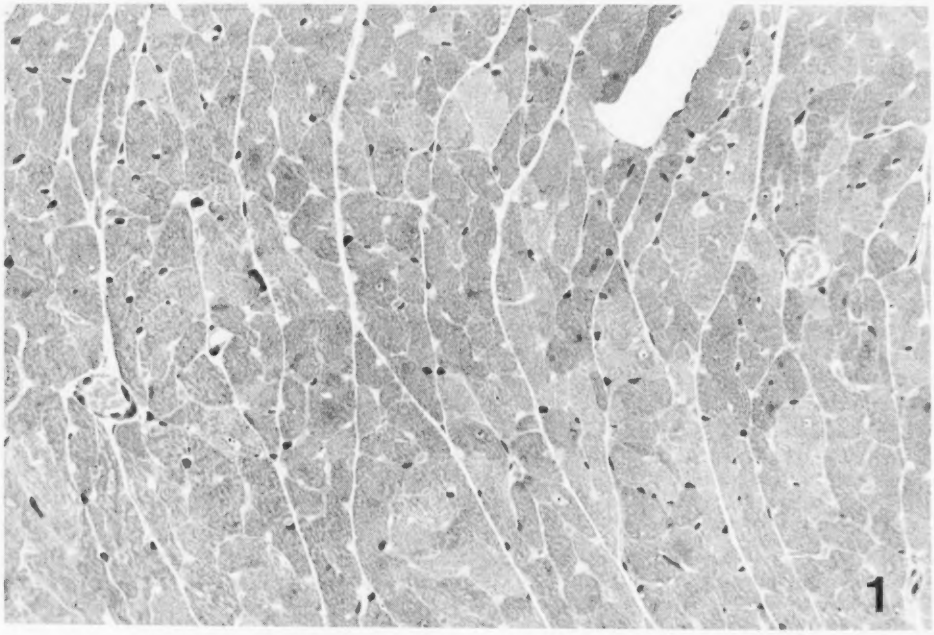

FIGURE 1.—Left ventricular myocardium from a control mouse (250 ×).

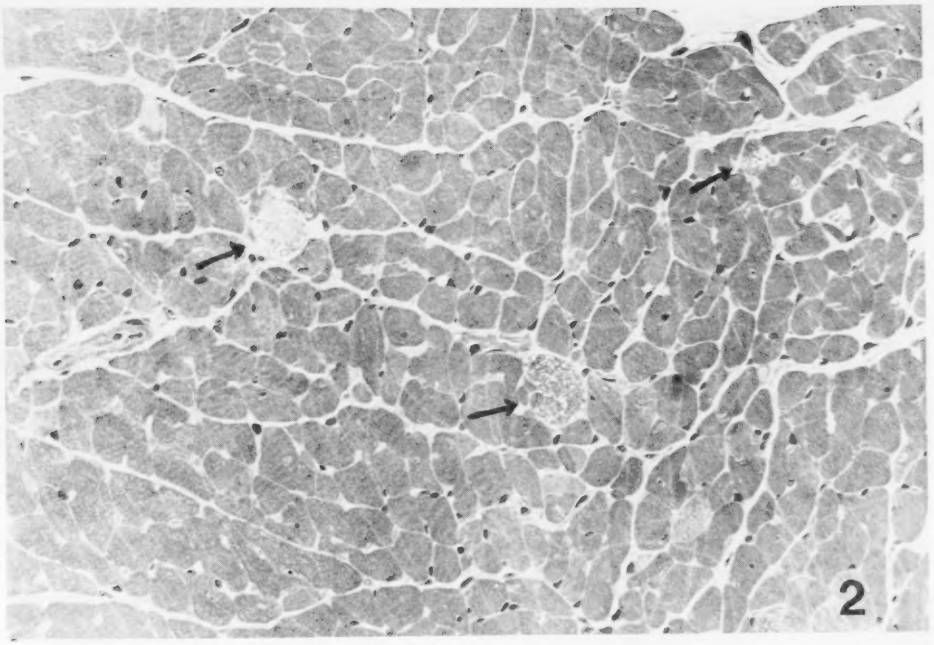

FIGURE 2.—Left ventricular myocardium from a mouse given Adriamycin at a total iv dose of 20 mg/kg (66.8 mg/m²); a single iv dose of 2 mg/kg was given twice a wk for 5 wks. 3 cells (arrows) exhibit fine cytoplasmic vacuolization. Severity degree = 1; extension degree = 1 (250 ×).

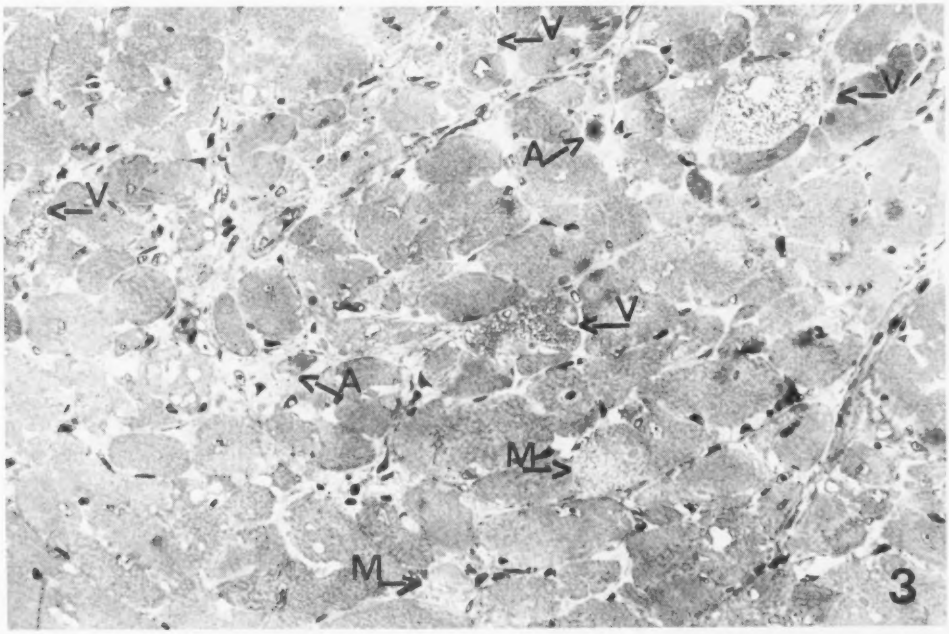

FIGURE 3.—Left ventricular wall from a mouse given Adriamycin at a total iv dose of 40 mg/kg (133.6 mg/m²); a single iv dose of 4 mg/kg was given twice a wk for 5 wks. Vacuolization (V), myolysis (M), myofiber atrophy (A), and focal interstitial fibrosis are evident. Severity degree = 2; extension degree = 3 (400 ×).

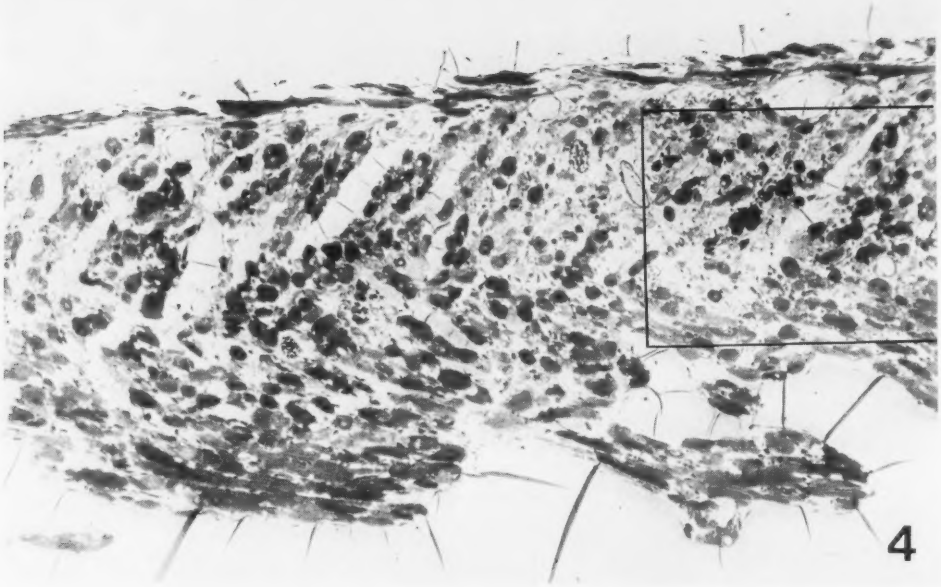

FIGURE 4.—Left ventricular wall of myocardium from a mouse given Adriamycin at a total iv dose of 40 mg/kg. Most of the cells are damaged. Severity degree = 2; extension degree = 5 (100 ×).

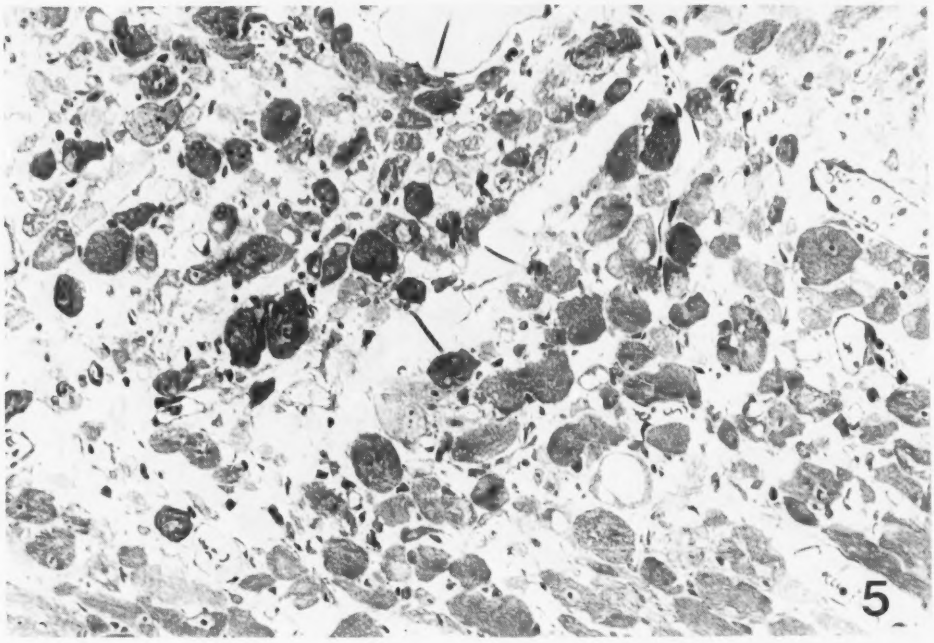

FIGURE 5.—Enlargement of squared area shown in fig 4. Severe and diffuse myocardial degeneration is shown with loss of myofibers, cellular atrophy, necrosis, and proliferation of connective tissue (400 ×).

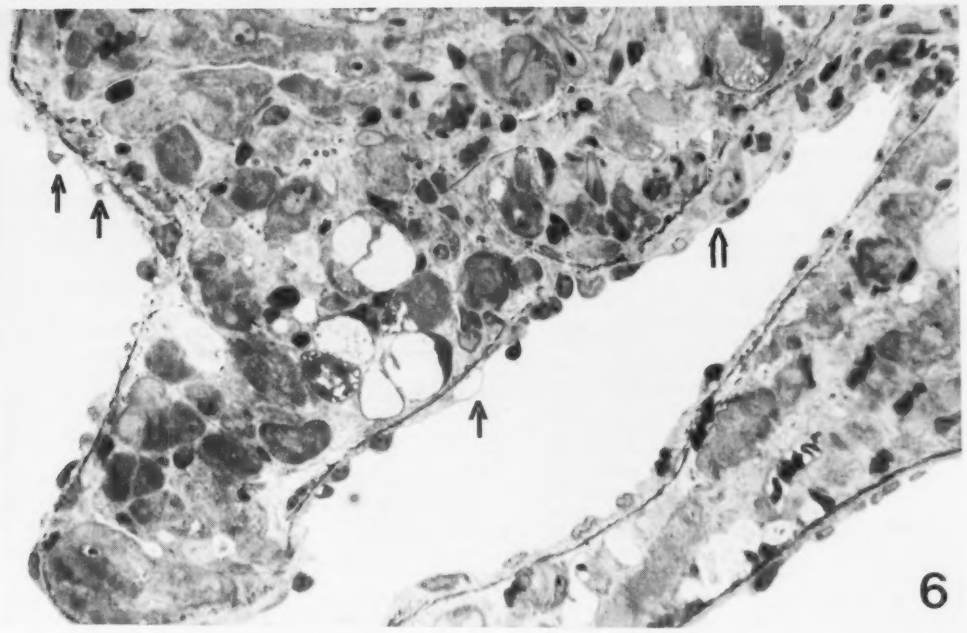

FIGURE 6.—Left atrium from a mouse given Adriamycin at a total iv dose of 40 mg/kg. Most of the cells show very severe lesions (atrophy, myolysis, large vacuolization, and necrosis). Thickening of the endocardium (large arrow), swollen endothelial cells (small arrows), and inflammatory cells are present. Severity degree = 2 (400 ×).

degree of extension. At higher doses more severe lesions appeared, such as loss of myofibers, atrophy, myolysis, and cell necrosis with connective tissue proliferation (severity 2). These lesions were generally accompanied by a more extensive involvement of the myocardium. In addition, swelling of single endocardial cells and thickening of the endocardium were observed in the atria. Ultrastructural examination of these lesions showed an increase in the number and extent of endocardial cell microvilli. Detailed data on atrial features will be reported elsewhere.

Toxic clinical symptoms were absent in all groups. Only one mouse, treated with the highest dose of Adriamycin, died after six injections. Body growth was not affected by Adriamycin at the doses of 1 and 2 mg/kg/day, whereas it was almost completely inhibited at the highest dose. This dose also caused minimal lesions in the spleen (follicular hypoplasia), kidneys (vacuolar degeneration of tubular epithelial cells), and liver (nuclear enlargement and focal cell necrosis).

Individual and mean scores of the quantitative heart evaluation for treated and control animals are reported in table 2, and the regression line is shown in figure 7. The MeCCD of Adriamycin is 36.4 mg/kg of body weight, with confidence limits of 31.5–43.6 mg/kg.

DISCUSSION

Mice repeatedly injected iv with Adriamycin twice a week for ten times, at doses equal to fixed fractions of the LD50, showed heart degenerative lesions similar to those described in man (10,11) and animals (2,3,6,12) after repeated treatment with anthracycline drugs. There is a significant correlation between the lesion scores and the doses, and the MeCCD for Adriamycin obtained with this method is 36.4 mg/kg (121.5 mg/m^2), which agrees with the initial cumulative cardiotoxic dose of 180 mg/m^2 found in man by Friedman et al (11). The confidence limits of the MeCCD are 31.5–43.6 mg/kg which allow differentiation between two drugs with a different cardiac toxicity of about 15%. The results reported herein were obtained in animals in apparent satisfactory condition; mortality and toxic degenerative lesions of other tissues were absent at the two lower doses and minimal at the highest dose.

This method avoids the significant problems encountered in rationally evaluating the cardiotoxic effects of anthracycline drugs and provides a practical and economic solution to common screening problems. The mouse test is carried out in vivo and thus allows one to take into account the unknown or uncertain role of me-

TABLE 2.—Quantitative evaluation of cardiac lesions, single and mean scores, and statistical analysis

	Single scores from Adriamycin cumulative doses (mg/kg) of—			
	0	10	20	40
	0	0.5	1.0	6.0
	0	0.0	3.0	4.0
	0	0.0	0.5	4.0
	0	0.0	2.0	6.0
	0	0.5	1.0	8.0
	0	0.5	2.0	4.0
	0	0.5	2.0	6.0
	0	0.5	6.0	6.0
	0	0.5	1.0	6.0
	0	0.0	1.0	6.0
	0	0.0	0.5	8.0
	0	0.0	1.0	8.0
	0	0.0	1.0	6.0
	0	0.0	2.0	4.0
	0	0.0	2.0	6.0
Mean	0	0.20	1.73	5.86

MeCCD = 36.4 mg/kg
Confidence limits (for $P = 0.95$) = 31.5–43.6 mg/kg
Regression line equation: $Y = -0.215 + 9.135 \log x$

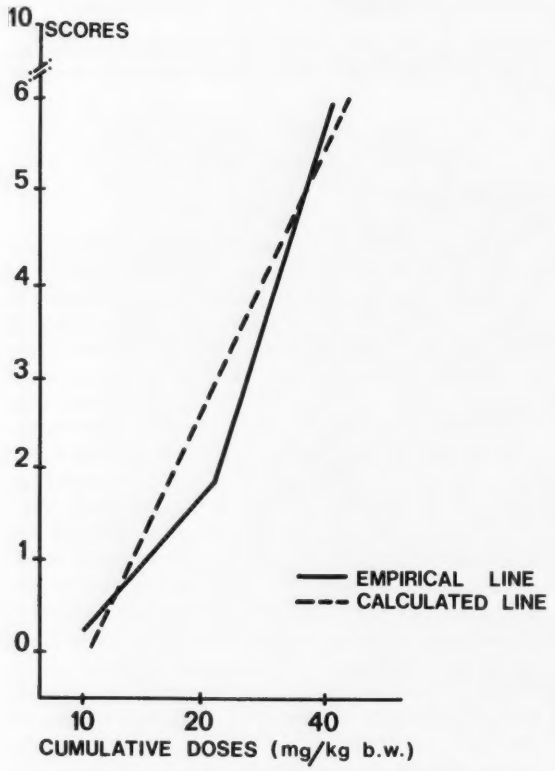

FIGURE 7.—Regression line of cardiotoxicity scores after doses of Adriamycin.

tabolites and the possible interference of other systemic effects of the drugs. Moreover, it directly evaluates cardiotoxicity through an extensive morphologic evaluatior of the heart. In fact, due to the focal character and the slow progression and evolution of the lesions, it is necessary to examine a large representative portion of the heart and to consider both the morphology and the extent of the alterations. Different analogs probably induce different patterns and topographic distributions of cardiac lesions. Moreover, the diagnosis of heart damage through biochemical or functional parameters is made difficult by the pathologic effects of the anthracycline drugs (11).

It is also important to obtain cardiac lesions by injecting relatively small doses iv in repeated cycles of treatment followed by breaks in treatment, because this schedule mimics the cumulative situation and the general conditions found in most of the experimental and clinical antitumor trials and allows full expression of the cardiac damage. Animals with cardiac lesions should be in satisfactory physical condition to eliminate the interference of important modifications in the pharmacokinetic behavior of Adriamycin resulting from serious damage of the liver and kidneys: these lesions are probable with the administration of single doses higher than one fifth of the LD50 as performed by others (7).

The use of at least three doses allows the statistical calculation of the median toxic effect, after a fixed treatment period, and standardizes the comparison of the absolute cumulative properties of different drugs. Finally, the use of a small animal minimizes drug requirement and the experimental cost. Only about 20 mg of Adria-

mycin was used for the entire test, and proportional quantities of other analogs would probably be sufficient, according to the respective LD50s.

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Therapy for Hepatocellular Cancer With Intrahepatic Arterial Adriamycin and 5-Fluorouracil Combined With Whole-Liver Irradiation: A Northern California Oncology Group Study[1,2]

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SUMMARY

Thirteen patients with hepatocellular cancer were treated with intrahepatic arterial Adriamycin and 5-fluorouracil combined with whole-liver irradiation. Six patients (46%) had an objective regression and five (38%) had stable disease. Symptomatic improvement was noted in 81% of the patients. The minimum median survival time was 5.0 months for those patients who responded. Toxicity was acceptable. This is an effective combination for the treatment of this especially aggressive and intractable form of cancer.

[Cancer Treat Rep 63:1885–1888, 1979]

Although hepatocellular cancer is uncommon in the US, it is the major malignant disease in parts of Africa and the Orient (1,2). There is no widely accepted, standard therapy for this particularly aggressive and intractable form of cancer. Surgical resection is appropriate in only a minority of patients and is rarely curative (3). Standard fraction radiation therapy alone is rarely effective (4). Systemic chemotherapy, most commonly with the fluoropyrimidines and more recently with Adriamycin (5–8), can induce responses, but these responses are usually not long-lasting. Some success with administration of these drugs via the hepatic artery has been documented (9–11), but this has often proven to be a cumbersome technique.

Concurrent administration of Adriamycin and 5-fluorouracil (5-FU) has been shown to potentiate radiation effects, and it is surprising that, to date, few attempts have been made to exploit these effects in the treatment of hepatocellular cancer (12). This study reports a phase I–II trial in which 13 patients with primary liver cancer were treated with intrahepatic arterial Adriamycin and 5-FU combined with rapid whole-liver irradiation. The results indicate that this regimen is an effective new approach in the therapy for primary liver cancer.

PATIENTS

The criteria for patient selection were as follows: (*a*) biopsy-confirmed hepatocellular carcinoma; (*b*) Karnofsky performance status of $\geq 50\%$; (*c*) hepatomegaly or disease measurable on radionuclide or computerized axial tomographic liver scan; (*d*) no previous Adriamycin or hepatic arterial chemotherapy; (*e*) no significant pre-existing cardiac disease that would preclude Adriamycin therapy; (*f*) pretherapy wbc count of $> 4000/mm^3$, platelet count of $\geq 100{,}000/mm^3$, and BUN of < 30 mg/100 ml; (*g*) estimated survival of > 4 weeks; and (*h*) informed consent.

The 13 patients entered in the study ranged in age from 28 to 74 years. There were ten males (four oriental, five white, and one black) and three females (all white). The evaluation of these patients before treatment is summarized in table 1. Two patients had had previous fluoropyrimidine chemotherapy.

Liver function abnormalities were noted in 11 of the 13 patients. Five patients had cirrhosis, four had elevated alpha-fetoprotein values, and two had hepatitis B antigenemia.

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TABLE 1.—Phase I–II trial: patient characteristics

Patient No.	Characteristic				
	Age	Race*	Sex	Karnofsky performance status (%)	Duration of disease before treatment (mos)
1	67	W	F	70	7
2	63	W	F	80	24
3	45	O	M	90	6
4	74	W	F	80	2
5	48	O	M	80	5
6	58	O	M	60	4
7	58	W	M	70	3
8	28	W	M	90	5
9	66	O	M	50	3
10	50	B	M	50	3
11	56	W	M	80	18
12	60	W	M	90	10
13	65	W	M	90	0.5

*W = white; O = oriental; B = black.

METHODS

Patients were evaluated before the initiation of therapy and daily during treatment with cbc and platelet counts and liver and renal function tests. Liver size was assessed by palpation and measured in the right midclavicular, subxiphoid, and left midclavicular vertical lines. Two observers made independent assessments of each dimension. Liver scans (either radionuclide or computerized axial tomographic) were also obtained at the beginning of therapy.

Chemotherapy was administered through the common hepatic artery via an indwelling catheter. This fluoroscopically monitored line was placed percutaneously into either the axillary or the femoral artery. The integrity of this catheter was maintained with 5% dextrose in 25% normal saline solution and 4000 units of heparin/liter, and its position was confirmed by routine abdominal roentgenograms. Each patient received 5-FU and Adriamycin through this arterial catheter. The 5-FU was continuously infused at a dose of 10 mg/kg/day beginning on the day of the first radiation treatment, interrupted only for bolus Adriamycin administration, and was continued until radiation therapy was completed (5–9 days). Adriamycin was given at a dose of 5 mg/m^2 1–2 hours before each radiation treatment (5–7 days). This Adriamycin dose was decreased by 50% when the patient had a serum bilirubin of 1.5–3.0 mg/100 ml and was decreased by 75% for a serum bilirubin of $>$ 3.0 mg/100 ml.

Radiation therapy was planned with arteriography image simulation and delivered with a 4 mev or ^{60}Co machine. The entire liver was treated at a rate of 300 rads/day to a total of 1500–2400 rads, over a period of 5–8 days. At least one third of the total renal volume was shielded from radiation. Therapy was delivered as planned despite marked hepatomegaly and treatment fields that frequently and unavoidably included portions of the lung bases, kidneys, bowel, and stomach.

Assessment of response was made at 1- and 2-week intervals. For the purpose of this study, complete assessment was made at 4 weeks after completion of therapy. An objective response was defined as a 50% decrease in lesions measured by scan or a 30% decrease in the sum of the three measurements of hepatomegaly. Regression was defined as a response sustained for a minimum of 4 weeks; tumors that had not regressed enough to meet this criterion, but had regressed to a lesser degree or had not changed, were considered stable. Progressive disease was defined as a $\geq$ 25% increase in those lesions that could be measured or evaluated. Additionally, improvement or worsening of clinical symptoms and liver function tests were assessed. After this treatment, prescribed for the purposes of the study, subsequent chemotherapy was administered at the discretion of the patient's primary physician.

RESULTS

The objective (measured) and subjective (symptomatic) responses noted in these patients are shown in table 2. Objective regressions were observed in six of 13 patients (46%) and lasted for 4 to 15+ months (median, 4 months). Stable disease was observed in five of 13 patients and lasted for 1+ to 7 months (median, 6 months). Subjective improvements occurred in nine of 11 patients complaining of hepatic pain, nausea, and early satiety.

TABLE 2.—Clinical benefits to 13 patients with hepatocellular cancer treated with intrahepatic arterial Adriamycin and 5-FU combined with whole-liver irradiation

Patient No.	Response	Duration of response (mos)	Duration of survival (mos)	Symptomatic benefit
1	Partial regression	7.5	9	+
2	Partial regression	15+	15+	+
3	Partial regression	4	11	No symptoms
4	Stable	7	9	+
5	Stable	6.5	8	+
6	Progression	—	1	No symptoms
7	Progression	1	2	–
8	Stable	5	5	+
9	Partial regression	4	4	+
10	Stable	6+	6+	+
11	Partial regression	5+	5+	+
12	Partial regression	4+	5+	+
13	Stable	1+	1+	–

TABLE 3.—Hematologic toxicity of intrahepatic arterial Adriamycin and 5-FU combined with whole-liver irradiation

	Nadir		
Patient No.	Wbc count (cells/mm^3)	Platelet count (cells/mm^3)	Hemoglobin level (g/100 ml)
1	3900	175,000	6.8
2	3300	111,000	8.9
3	5400	263,000	8.2
4	4000	102,000	10.2
5	4400	110,000	11.0
6	2300	150,000	10.1
7	7000	320,000	8.5
8	4700	79,000	12.0
9	2100	175,000	10.2
10	4800	113,000	10.6
11	3400	55,000	12.0
12	3000	145,000	11.0
13	3900	153,000	7.5

Two of 13 patients had progressive disease in the first 4 weeks after therapy. The responders had a minimum mean survival of 8.2 months and a minimum median survival of 5 months. Biochemical improvement in liver function tests occurred in seven of 11 patients.

Toxicity and Side Effects

Nausea and vomiting were frequent but generally mild during treatment and were severe in only one patient. No pronounced alopecia was observed in any patient. Fever occurred in nine previously afebrile patients. In three patients, transient, mild worsening of liver function tests was observed. Hematologic toxicity was generally mild and is shown in table 3. The median nadir wbc count was 3900/mm^3 (range, 2100–7000/mm^3). The median nadir platelet count was 113,000/mm^3 (range, 55,000–320,000/mm^3). Treatment-associated anemia was seen in five of 13 patients, with a median nadir hemoglobin level of 8.9 g/100 ml (range, 6.8–12 g/100 ml). No catheter-associated infections, emboli, or bleeding episodes were observed, nor was cardiac toxicity observed.

DISCUSSION

Although hepatocellular carcinoma can metastasize widely to the lungs and abdominal viscera, the liver remains the site of gravest clinical consequence. Most patients die from hepatic failure, portal hypertension, and bleeding. This disease is usually rapidly fatal; ≤ 6 months is the median survival for the untreated patient (1,3). Therefore, to be beneficial a treatment program must be both vigorous and rapidly effective.

Chemotherapy trials have generally not been successful in providing subjective or objective benefits for patients. Therapy with 5-FU or other antimetabolites has resulted in occasional responses, but little substantial improvement in survival of patients has been reported. In 1975, Olweny and co-workers (7) reported that 11 of 11 evaluable patients from Uganda responded to Adriamycin, with a median survival of 8 months. More recently, Johnson et al (13) treated 44 patients with Adriamycin and reported an objective response rate of 32% and a median survival time of slightly > 3 months. The Eastern Cooperative Oncology Group evaluated four chemotherapy programs including those using 5-FU, nitrosoureas, and Adriamycin in an extensive prospective clinical trial. Only 6% of 168 patients had an objective response. The median survival times ranged from 5 to 24 weeks (8). Sporadic reports of hepatic intra-arterial

5-FU have indicated some promise, but the effectiveness of this treatment has not been substantiated in well-controlled trials. Combinations of drugs may be more beneficial than individual single agents, but again, data in support of this speculation have been inadequate (12–14).

Most reported trials of combined radiation therapy and chemotherapy have yielded discouraging results, with median survival times of between 7 and 17 weeks. These poor responses are at least partially a result of the long period of time required to deliver the radiation therapy. In the series reported by Cochrane et al (4), 21 days were necessary for radiation therapy, and chemotherapy was not initiated until 60 days thereafter. Hence, few patients were alive to receive the potential benefits from chemotherapy.

For this reason, we chose to combine rapid-fraction radiation therapy with intrahepatic arterial 5-FU and Adriamycin and use them aggressively. We concentrated our initial efforts in a phase I study to determine the toxic side effects of this regimen. Our concerns about possible acute and subacute toxic effects proved unfounded and we determined that 2100 rads plus intra-arterial 5-FU (10 mg/kg/day) and Adriamycin (5 mg/m^2/day) could be safely administered and tolerated.

We recognize that in this preliminary study our sample size was small and our followup was limited; however, we were nonetheless impressed by the number of objective (46%) and subjective (81%) responses we observed, and the duration of both these benefits and patient survival. Results of this pilot study suggest that combined chemotherapy plus irradiation should be seriously considered as an "induction" therapy for patients with hepatocellular cancer. After this treatment, higher doses of radiation could be delivered to smaller volumes of the involved portions of liver, along with "maintenance" systemic chemotherapy. We are currently initiating a prospective controlled clinical trial to assess the relative effectiveness of these proposed treatment options.

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Schedule-Dependent Synergism of Methotrexate and Vincristine Against Murine L1210 Leukemia [1,2]

P. L. Chello,* F. M. Sirotnak, D. M. Dorick, and D. M. Moccio [3]

SUMMARY

$BD2F_1$ mice were inoculated with 10^6 L1210 murine lymphocytic leukemia cells and treated simultaneously with methotrexate and vincristine or with methotrexate followed by vincristine ≥ 24 hours later. Four to six doses of methotrexate, 48 mg/kg ip, administered every 4 days beginning on Day 1 resulted in a 168%–228% increase in lifespan, while vincristine, at 0.5 mg/kg ip, given on the same schedule until death (two or three doses) gave only a 37% increase in lifespan. Simultaneous administration of both agents resulted in a therapeutic effect which was approximately additive. When vincristine was given 24 hours or 24 and 72 hours after the methotrexate, a further increase (70%–100%) in lifespan over that expected from an additive effect and long-term survivors (> 90 days) were obtained. Synergism between the two agents and long-term survivors were also seen with higher methotrexate concentrations (72 or 96 mg/kg) given in four or five courses. If therapy was initiated on Day 2 when the peritoneal tumor burden was approximately 2×10^7 cells, the combination of methotrexate with delayed vincristine still resulted in an increased therapeutic effect over that obtained with either drug alone, or that expected on an additive basis.

[Cancer Treat Rep 63:1889–1894, 1979]

In 1973, Zager et al (1) and Fyfe and Goldman (2) reported in vitro observations showing that the vinca alkaloid, vincristine, altered the steady-state level of methotrexate accumulation in L1210 murine lymphocytic leukemia cells and in Ehrlich ascites tumor cells respectively. Other investigators later showed that the vinca alkaloid produced a similar alteration in methotrexate transport by human tumor cells (3,4). In the original report (1) on the effect of vincristine on methotrexate transport by L1210 tumor cells, data were also presented suggesting that the administration of vincristine shortly before methotrexate to mice bearing L1210 leukemia resulted in therapeutic synergism and long-term survivors. On the basis of this report, administration of vincristine prior to methotrexate was incorporated into clinical protocols involving high-dose methotrexate therapy (5–7).

Subsequent studies reported from this laboratory (8) and elsewhere (9) have shown that vincristine neither alters methotrexate pharmacokinetics in tumor cells in situ nor is synergistic when administered prior to or together with methotrexate. However, in an extension of our own studies (8), it was found that the combination of methotrexate and vincristine was therapeutically synergistic against the L1210 leukemia if the vinca alkaloid was administered at least 8 hours after the folate analog. The magnitude of the synergism observed in these experiments was proportionally greater as the interval between the two agents was increased to at least 24 hours. The current report presents additional results obtained during therapy against L1210 leukemia incorporating different doses of methotrexate and a varying number of courses, and shows that certain schedules employing this combination are partially curative.

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TABLE 1.—Treatment on Day 1 of L1210 leukemia with 48 mg/kg of methotrexate (MTX) ip and 0.5 mg/kg of vincristine (VCR) ip

MTX* (mg/kg)	VCR (mg/kg)	Interval between MTX and VCR therapy (hrs)	No. of courses†	Total mice‡	MST§ ± SD (days)	ILS (%)	Final weight ± SD (g)	90-day survivors¶
—	—	—	—	6 × 5	6.5 ± 0.5	—	22.7 ± 1.3	0/30
—	0.5**	—	2–3	6 × 5	8.9 ± 0.8	37	22.8 ± 1.6	0/30
48	—	—	4	5 × 5	17.4 ± 2.5	168	20.6 ± 2.0	0/25
			5	6 × 5	18.7 ± 5.3	188	20.0 ± 2.9	0/30
			6	3 × 5	21.3 ± 3.2	228	21.2 ± 2.9	0/15
48	0.5	Given together	4	3 × 5	20.2 ± 3.9	211	20.3 ± 2.6	0/15
			5	3 × 5	21.8 ± 5.0	235	17.6 ± 2.8	0/15
			6	3 × 5	21.1 ± 5.3	225	15.4 ± 2.1	0/15
48	0.5	24	4	5 × 5	24.3 ± 7.0	274	19.6 ± 2.7	0/25
			5	5 × 5	27.6 ± 12.3	325	17.6 ± 5.9	2/25
			6	3 × 5	24.8 ± 8.2	281	17.4 ± 4.2	3/15
48	0.5	48	4	2 × 5	26.7 ± 13.3	311	19.3 ± 4.3	0/10
			5	2 × 5	22.7 ± 8.5	249	16.9 ± 4.1	1/10
			6	2 × 5	26.1 ± 6.1	302	16.1 ± 2.0	1/10
48	0.5	72	4	4 × 5	21.4 ± 5.4	229	21.5 ± 2.2	0/20
			5	6 × 5	25.8 ± 8.6	297	19.8 ± 2.8	2/30
			6	3 × 5	26.9 ± 11.6	314	20.5 ± 4.8	0/10
48	0.5	24 and 48††	4	3 × 5	19.4 ± 8.3	198	16.5 ± 3.5	0/15
			5	2 × 5	16.4 ± 2.1	152	15.1 ± 1.0	1/10
			6	2 × 5	21.5 ± 3.7	230	14.1 ± 0.6	1/10
48	0.5	48 and 72††	4	2 × 5	21.8 ± 17.0	235	18.9 ± 7.3	0/10
			5	2 × 5	15.8 ± 9.4	143	16.8 ± 4.5	0/10
			6	2 × 5	14.8 ± 5.4	127	14.8 ± 0.8	0/10
48	0.5	24 and 72††	4	5 × 5	20.0 ± 9.0	208	17.9 ± 4.8	2/25
			5	4 × 5	26.4 ± 13.1	306	19.5 ± 3.9	4/20
			6	3 × 5	20.1 ± 4.9	209	15.6 ± 4.9	1/15

*Given ip every 4 days beginning on Day 1.
†No. of times MTX (with or without VCR) was administered.
‡No. of experiments × No. of mice per experiment.
§Mean survival time (90-day survivors not included).
¶No. of animals surviving 90 days after cessation of therapy/No. treated.
**Given on Day 1 and every 4 days thereafter until death.
††1 dose of 0.5 mg/kg at each time.

MATERIALS AND METHODS

Methotrexate (provided by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, as the sodium salt) and vincristine (from Eli Lilly and Co, Indianapolis, IN) were prepared in sterile distilled water. L1210 tumor cells (line V) were maintained (10) by ip transplantation in female $BD2F_1$ mice (C57BL female × DBA_2 male obtained from A. R. S. Sprague/Dawley, Madison, WI). For therapy experiments, female $BD2F_1$ mice (initial weight 20.0 ± 0.5 g) received ip inoculations of 10^6 L1210 tumor cells on Day 0 and were randomly distributed between control (untreated) and treated groups. Untreated animals died with well-developed ascites within 6–7 days. Treated animals received each drug separately as a single injection ip beginning at 24 hours (Day 1) or 48 hours (Day 2) after transplantation. Animals were maintained with Purina laboratory chow and water ad libitum.

RESULTS AND DISCUSSION

Our earlier communication (8) described preliminary evidence of the schedule-dependent synergism between methotrexate and vincristine against the L1210 leukemia. These results indicated that the most effective therapeutic action of this combination was obtained when vincristine administration was delayed for at least 24 hours after methotrexate. By comparison, the combination was least effective when both drugs were given simultaneously.

Consequently, the experiments to be described here compared only schedules using the simultaneous administration of these agents with those using an interval between the administration of each agent of $\geq$ 24 hours. In order to be consistent with our previously published study (8), we used a therapeutic regimen in which methotrexate was administered every 4 days for a total of four to six doses. The LD10 dose calculated for this schedule during the current experiments was 53 mg/kg. This was derived by probit analyses of survival time data when mice were administered a total of five doses (every 4 days $\times$ 5). The results shown in table 1 were obtained when mice inoculated with 10^6 L1210 cells were treated with 48 mg/kg of methotrexate with or without 0.5 mg/kg of vincristine beginning 1 day after implantation. Control animals survived an average of 6.5 $\pm$ 0.5 days. Four to six courses of methotrexate alone resulted in a 168%–228% ILS and no long-term survivors. Vincristine alone given every 4 days starting at the same time after tumor implantation and continued until death of the animals (a total of two or three doses) resulted in only a 37% ILS. The simultaneous administration of both agents resulted in an ILS which was approximately additive. In contrast, the administration of vincristine 24 hours after methotrexate resulted in an ILS of 274%–325% after four to six courses and long-term survivors with five or six courses (five long-term survivors among 40 animals). When vincristine was administered with a delay of 48 or 72 hours after methotrexate, an antitumor effect comparable to that seen after a 24-hour delay was observed, and some long-term survivors were again obtained.

Because our previous study (8) showed that the synergistic effect of this drug combination was related to the dose of vincristine as well as to the time of administration, we sought to increase the effect of this vinca alkaloid by the administration of two sequential doses of 0.5 mg/kg. The administration of vincristine at 24 and 48 or 48 and 72 hours after methotrexate proved to be toxic as indicated by the severe weight loss. A schedule using two doses at 24 and 72 hours after the folate analog, ie, separated by an interval of 48 hours, was more tolerated, but the ILS and the number of long-term survivors obtained with four or five courses were comparable to those seen when a single dose of vincristine was given 24 hours after methotrexate. Six courses of therapy on this schedule, however, also appeared to be toxic.

Results obtained with other doses of methotrexate and delayed administration of vincristine (0.5 mg/kg) are given in table 2. In these experiments, we sought to determine the potential for further increases in the therapeutic effects of this combination by using higher doses of methotrexate for a smaller number of courses. As in the experiment described in table 1, therapy with methotrexate was initiated 24 hours after tumor cell implant. Administration of vincristine at 24 or 72 hours after either 72 or 96 mg/kg of methotrexate for a total of four or five courses resulted in an apparent synergism and, in some cases, long-term survivors were obtained. With few exceptions, these results were comparable to those obtained with 48 mg/kg of methotrexate after five or six courses (table 1). When vincristine was administered at both 24 and 72 hours after 72 mg/kg of methotrexate, the ILS obtained was somewhat greater than that obtained with a single dose of vincristine delayed either 24 or 72 hours but there were no long-term survivors. This latter result is in contrast to that seen with the same schedule employing 48 mg/kg of methotrexate. The schedule employing 96 mg/kg of methotrexate followed by vincristine at 24 and 72 hours was toxic.

The results presented in tables 1 and 2 were obtained after therapy was initiated 24 hours after tumor implantation when the intraperitoneal tumor cell number was approximately 4 $\times$ 10^6 cells.[4] Further evidence of the potential value of this combination regimen (methotrexate with delayed vincristine) against more advanced disease was sought in other experiments shown in tables 3 and 4. In these experiments, treatment was delayed until 2 days after tumor implantation when the intraperitoneal tumor cell number was approximately 2 $\times$ 10^7 cells.[4] The results of these experiments (tables 3 and 4) show that those schedules found to be most effective against the early leukemia were also most effective against the more advanced leukemia. The overall effect on lifespan of 48 mg/kg of methotrexate using any schedule with or without vincristine was comparable in each case; however, no long-term survivors were obtained during treatment of the more advanced leukemia. The use of 72 or 96 mg/kg of methotrexate with delayed vincristine increased the therapeutic effect as compared to that observed with 48 mg/kg. In some cases, an equivalent therapeutic gain was obtained with fewer courses of therapy. Long-term survivors were found only when vincristine was given at both 24 and 72 hours after either level of methotrexate (five courses).

These results confirm the schedule-dependent nature of the synergism of this drug combination against this murine leukemia. In addition, information has also been derived relating to the optimization and effectiveness of this combination in this tumor model. First, the time of vincristine administration after methotrexate appears to be flexible as long as a sufficient interval is allowed to elapse prior to the administration of the vinca alkaloid. Although the results seemed to be best when an interval of 24 hours was used, a further delay in vincristine administration was also highly effective. Second, the total amount of vincristine given can be increased by the administration of sequential doses providing that they

[4]Measured after peritoneal lavage with phosphate buffered isotonic saline.

TABLE 2.—Treatment on Day 1 of L1210 leukemia with 72 or 96 mg/kg of methotrexate (MTX) ip and 0.5 mg/kg of vincristine (VCR) ip

MTX* (mg/kg)	VCR (mg/kg)	Interval between MTX and VCR therapy (hrs)	No. of courses†	Total mice‡	MST§ ± SD (days)	ILS (%)	Final weight ± SD (g)	90-day survivors¶
—	—	—	—	6 × 5	6.5 ± 0.5	—	22.7 ± 1.3	0/30
—	0.5**	—	2–3	6 × 5	8.9 ± 0.8	37	22.8 ± 1.6	0/30
72	—	—	4	3 × 5	17.3 ± 3.4	166	21.2 ± 1.7	0/15
	—	—	5	4 × 5	16.5 ± 10.4	154	21.5 ± 1.8	0/20
72	0.5	24	4	3 × 5	22.5 ± 13.9	246	21.5 ± 2.0	2/15
			5	3 × 5	27.0 ± 12.0	315	21.2 ± 2.4	2/15
72	0.5	72	4	3 × 5	27.3 ± 8.1	320	21.2 ± 4.3	0/15
			5	4 × 5	27.3 ± 12.1	320	18.1 ± 5.2	0/20
72	0.5	24 and 72††	4	3 × 5	28.4 ± 14.4	337	19.0 ± 2.9	0/15
			5	2 × 5	30.2 ± 13.5	365	17.9 ± 4.6	0/10
96	—	—	4	3 × 5	14.6 ± 4.5	125	21.3 ± 2.5	0/15
			5	4 × 5	21.2 ± 4.5	226	19.6 ± 3.2	0/20
96	0.5	24	4	3 × 5	27.2 ± 7.4	318	21.1 ± 2.4	2/15
			5	3 × 5	31.2 ± 9.1	380	21.9 ± 1.6	2/15
96	0.5	72	4	3 × 5	20.0 ± 10.0	208	19.5 ± 3.6	2/15
			5	3 × 5	27.6 ± 9.4	324	20.1 ± 3.5	0/15
96	0.5	24 and 72††	4	3 × 5	21.1 ± 12.7	225	18.6 ± 3.4	1/15
			5	3 × 5	19.5 ± 5.0	200	15.4 ± 2.3	0/15

*Given every 4 days beginning on Day 1.
†No. of times MTX (with or without VCR) was administered.
‡No. of experiments × No. of mice per experiment
§Mean survival time (90-day survivors not included).
¶No. of animals surviving 90 days after cessation of therapy/No. treated.
**Given on Day 1 and every 4 days thereafter until death.
††1 dose of 0.5 mg/kg at each time.

TABLE 3.—Treatment on Day 2 of L1210 leukemia with 48 mg/kg of methotrexate (MTX) ip and 0.5 mg/kg of vincristine (VCR) ip

MTX* (mg/kg)	VCR (mg/kg)	Interval between MTX and VCR therapy (hrs)	No. of courses†	Total mice‡	MST§ ± SD (days)	ILS (%)	Final weight ± SD (g)
—	—	—	—	12 × 5	6.6 ± 0.7	—	22.0 ± 4.1
—	0.5¶	—	2–3	12 × 5	9.4 ± 0.8	42	21.2 ± 1.8
48	—	—	4	9 × 5	15.5 ± 3.4	135	20.4 ± 1.5
			5	11 × 5	16.7 ± 3.4	153	20.4 ± 2.1
			6	2 × 5	15.3 ± 4.2	132	21.4 ± 2.8
48	0.5	Given together	4	3 × 5	18.4 ± 3.6	178	17.8 ± 2.7
			5	3 × 5	20.2 ± 4.4	206	16.7 ± 3.1
			6	3 × 5	16.0 ± 5.1	142	15.6 ± 2.7
48	0.5	24	4	8 × 5	21.2 ± 4.3	221	20.3 ± 2.2
			5	8 × 5	25.0 ± 4.5	279	20.2 ± 1.9
			6	4 × 5	30.9 ± 4.4	368	18.6 ± 1.6
48	0.5	48	4	3 × 5	24.8 ± 3.5	276	20.3 ± 1.3
			5	3 × 5	24.4 ± 4.3	270	17.8 ± 3.5
			6	3 × 5	25.5 ± 8.9	286	18.1 ± 2.9
48	0.5	72	4	6 × 5	21.1 ± 4.0	220	20.6 ± 2.3
			5	8 × 5	22.3 ± 5.1	238	19.0 ± 3.8
			6	4 × 5	26.1 ± 4.6	295	18.4 ± 2.7
48	0.5	24 and 48**	4	3 × 5	19.3 ± 8.4	192	16.0 ± 2.1
			5	2 × 5	20.4 ± 3.1	209	14.3 ± 1.3
			6	3 × 5	21.7 ± 6.7	229	16.0 ± 1.4
48	0.5	48 and 72**	4	6 × 5	14.5 ± 5.3	120	14.9 ± 1.4
			5	2 × 5	18.5 ± 3.8	180	15.1 ± 1.1
			6	1 × 5	14.8 ± 11.9	124	15.8 ± 2.5
48	0.5	24 and 72**	4	7 × 5	20.0 ± 8.9	203	17.5 ± 3.1
			5	4 × 5	28.5 ± 7.9	332	19.3 ± 2.3
			6	4 × 5	32.2 ± 11.8	388	18.5 ± 5.6

*Given every 4 days beginning on Day 2.
†No. of times MTX (with or without VCR) was administered.
‡No. of experiments × No. of mice per experiment.
§Mean survival time.
¶Given on Day 2 and every 4 days thereafter until death.
**1 dose of 0.5 mg/kg at each time.

TABLE 4.—Treatment on Day 2 of L1210 leukemia with 72 or 96 mg/kg of methotrexate (MTX) ip and 0.5 mg/kg of vincristine (VCR) ip

MTX* (mg/kg)	VCR (mg/kg)	Interval between MTX and VCR therapy (hrs)	No. of courses†	Total mice‡	MST§ ± SD (days)	ILS (%)	Final weight ± SD (g)
—	—	—	—	12 × 5	6.6 ± 0.7	—	22.0 ± 4.1
—	0.5¶	—	2–3	12 × 5	9.4 ± 0.8	42	21.2 ± 1.8
72	—	—	4	6 × 5	15.7 ± 4.2	138	19.7 ± 2.7
			5	8 × 5	19.3 ± 3.5	192	20.4 ± 3.7
72	0.5	24	4	6 × 5	22.9 ± 3.7	246	20.5 ± 1.7
			5	6 × 5	24.4 ± 6.6	270	19.5 ± 2.1
72	0.5	72	4	3 × 5	19.0 ± 3.8	188	19.1 ± 3.7
			5	5 × 5	21.2 ± 5.0	221	18.1 ± 2.9
72	0.5	24 and 72**	4	3 × 5	25.7 ± 6.6	289	19.8 ± 3.2
			5	3 × 5	25.8 ± 11.0††	291	18.9 ± 6.0
96	—	—	4	6 × 5	17.4 ± 4.3	164	20.3 ± 4.3
			5	6 × 5	20.1 ± 3.9	204	18.6 ± 3.7
96	0.5	24	4	6 × 5	24.4 ± 4.6	270	21.1 ± 2.3
			5	6 × 5	25.9 ± 9.9	292	18.8 ± 4.3
96	0.5	72	4	3 × 5	24.6 ± 5.3	272	21.2 ± 1.7
			5	3 × 5	26.7 ± 5.0	304	16.1 ± 5.9
96	0.5	24 and 72**	4	2 × 5	28.0 ± 7.6	324	19.1 ± 2.9
			5	2 × 5	28.1 ± 7.6‡‡	326	17.3 ± 3.6

*Given ip every 4 days beginning on Day 2.
†No. of times MTX (with or without VCR) was administered.
‡No. of experiments × No. of mice per experiment.
§Mean survival time.
¶Given on Day 2 and every 4 days thereafter until death.
**1 dose of 0.5 mg/kg at each time.
††2 90-day survivors not included in average.
‡‡1 90-day survivor not included in average.

are not timed too closely. Third, methotrexate doses below the LD10 level on an optimum schedule with vincristine are substantially more effective than higher doses of methotrexate alone, since a larger number of courses of the combination are tolerated. The data also show that the optimum schedule for this combination of drugs will cure mice bearing a substantial number of tumor cells, whereas either drug alone will not. Finally, this regimen is quite effective against mice bearing a more advanced leukemia. On the basis of these considerations, this combination of agents used in the schedule shown to be optimum during these studies may have clinical potential.

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Antineoplastic Effect of Orally Administered 1-Alkyl Carbamoyl Derivatives of 5-Fluorouracil on Sc Implanted Lewis Lung Carcinoma and B16 Melanoma [1,2,3]

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SUMMARY

The antitumor activity of 1-alkyl carbamoyl derivatives of 5-fluorouracil against Lewis lung carcinoma and B16 melanoma by long-term oral administration was examined. The 1-hexyl and 1-phenethyl carbamoyl-5-fluorouracil derivatives were markedly active against early Lewis lung carcinoma among the derivatives tested. These compounds were not markedly active against advanced Lewis lung carcinoma but did show acceptable activity. Increases in lifespan in mice with early Lewis lung carcinoma at optimal doses of 1-hexyl and 1-phenethyl carbamoyl-5-fluorouracil were 98% and 78% respectively. In advanced Lewis lung carcinoma, the 1-hexyl derivative was active by either intermittent or daily administration, but the 1-phenethyl derivative was active only by daily administration. Lung metastases were not inhibited by optimal doses of the 1-hexyl derivative but were completely inhibited by the 1-phenethyl derivative. The 1-hexyl derivative was also active against B16 melanoma and the increase in lifespan at optimal doses was 27%. As a result, 1-hexyl carbamoyl-5-fluorouracil was found to be the most active derivative against early Lewis lung carcinoma and B16 melanoma. However, 1-phenethyl carbamoyl-5-fluorouracil was the most active derivative against advanced Lewis lung carcinoma by daily administration and this compound completely inhibited lung metastases, while 5-fluorouracil and cyclophosphamide did not inhibit lung metastases.

[Cancer Treat Rep 63:1895–1899, 1979]

Many of the 1-alkyl carbamoyl-5-fluorouracils, which were synthesized by Ozaki et al (1), were markedly active in the L1210 leukemia system (2). Among them, 1-hexyl carbamoyl-5-fluorouracil showed the highest therapeutic ratio (2). Moreover, several alkyl carbamoyl derivatives of 5-fluorouracil (5-FU) were also found to be markedly active against C1498 leukemia, ascites sarcoma 180, Ehrlich ascites carcinoma, Nakahara-Fukuoka sarcoma, and adenocarcinoma 755 (3). Among them, 1-methyl, 1-ethyl, 1-hexyl, 1-octyl, 1-isopropyl, and 1-phenethyl carbamoyl derivatives of 5-FU showed a broader spectrum of activity in the above six mouse tumor systems than either 5-FU or 1-(2-tetrahydrofuryl)-5-fluorouracil (FT) when given by oral administration.

In this report, the antitumor activity of the 1-alkyl carbamoyl derivatives of 5-FU against slow-growing tumors, namely, Lewis lung carcinoma and B16 melanoma, was examined by long-term oral administration. These solid tumors are refractory to most established chemotherapeutic agents, especially antimetabolites (4).

MATERIALS AND METHODS

Animals and tumors.—Test animals were male BDF_1

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[3]1-Alkyl carbamoyl-5-fluorouracil and 1-(2-tetrahydrofuryl)-5-fluorouracil were kindly supplied by Mitsui Pharmaceuticals, Inc, Tokyo, Japan.

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mice weighing 20–24 g. Lewis lung carcinoma (10^5 viable cells) or B16 melanoma (0.05 ml of a 33% tumor brei) was implanted sc on Day 0.

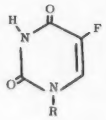
Compounds.—1-Alkyl carbamoyl-5-fluorouracils, 5-FU, FT, and cyclophosphamide were used. The chemical structures of the 1-alkyl carbamoyl-5-fluorouracils are shown in table 1.

Drug treatment.—Test compounds, suspended in 0.5% carboxymethyl cellulose solution as vehicle, were administered orally in a volume of 0.01 ml/g of mouse body weight. All compounds were prepared immediately before administration. Treatment for early tumors was started 24 hours after the tumor inoculation with the compound administered three times a week for 4 weeks. Moreover, the compounds which showed marked antitumor activity in the first experiment were tested in other schedules (three times a week or daily administration, starting on Day 14 after tumor implantation until death).

Evaluation of antitumor activity.—Six mice in each group, either test or control, were used. The growth of sc implanted Lewis lung carcinoma was monitored by caliper measurements of perpendicular diameters. Tumor weight was calculated in mg by the formula for volume of a prolate spheroid, assuming ½ × (major diameter in mm) × (minor diameter^2 in mm^2). Tumors were measured twice a week. Median survival time (median day of death) relative to untreated control groups was determined for evaluation of drug activity (5).

TABLE 1.—Chemical structure of 5-FU derivatives

Compound No.	–R
I	–H
II	
III	–CONHCH$_3$
IV	–CONHCH$_2$CH$_3$
V	–CONHCH$_2$CH$_2$CH$_2$CH$_2$CH$_3$
VI	–CONHCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$
VII	–CONHCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$
VIII	–CONHCH$_2$
IX	–CONHCH$_2$CH$_2$



RESULTS

Effect of 1-Alkyl Carbamoyl Derivatives of 5-FU on Early Lewis Lung Carcinoma and B16 Melanoma

As shown in table 2, most of these alkyl carbamoyl derivatives of 5-FU were active (ie, at least 25% increase in lifespan [ILS] in one experiment) against early Lewis lung carcinoma. The 1-hexyl and 1-phenethyl carbamoyl derivatives of 5-FU were especially markedly active against this tumor. In this experiment, the maximum ILSs of the 1-hexyl and 1-phenethyl derivatives were 97% and 78% using treatments given three times a week with 200 and 100 mg/kg respectively. Both compounds were as effective as cyclophosphamide, which was used as a positive control. However, the 1-methyl and 1-ethyl carbamoyl derivatives of 5-FU, which showed the most broad-spectrum activity in rapidly growing tumor systems (3), were not as active against Lewis lung carcinoma. 1-Octyl carbamoyl-5-fluorouracil was as active as FT, but 5-FU was not effective in the tumor by oral administration. Optimal doses of the 1-hexyl and 1-phenethyl derivatives were 200 and 100 mg/kg/day respec-

TABLE 2.—Antitumor activity of 5-FU derivatives on survival time in BDF$_1$ mice bearing sc Lewis lung carcinoma

Experiment No.	Compound No.	Dose* (mg/kg/day)	Median day of death (range)	ILS (%)
1	Control	—	31.5 (23–36)	—
	I	20	39.0 (29–46)	24
		30	36.5 (33–51)	16
	II	100	32.0 (27–40)	2
		200	45.0 (35–59)	43
		300	36.0 (29–64)	14
	III	30	42.5 (21–47)	35
		50	43.0 (27–73)	37
		100	19.0 (14–21)	–40
	IV	30	35.5 (27–38)	13
		50	44.0 (36–64)	40
		100	25.5 (19–41)	–19
	V	100	44.0 (39–67)	40
	VI	100	37.0 (28–48)	17
		200	62.0 (46–94)	97
		300	20.0 (17–21)	–37
	VII	200	47.0 (34–57)	49
		300	43.0 (33–57)	37
	VIII	50	43.0 (33–69)	37
		100	27.0 (22–35)	–10
	IX	50	33.0 (20–37)	5
		100	56.0 (52–64)	78
		200	20.0 (18–41)	–37
	Cyclophosphamide	30	60.0 (40–62)	90
2	Control	—	28.0 (20–36)	—
	VI	200	52.5 (30–59)	88
	IX	100	48.0 (40–55)	71

*Drugs were administered orally 3 times a wk for 4 wks beginning on Day 1 after tumor implantation.

TABLE 3.—Antitumor activity of 5-FU derivatives on survival time in BDF_1 mice bearing sc B16 melanoma

Compound No.	Dose* (mg/kg/day)	Median day of death (range)	ILS (%)
Control	—	30.0 (23–41)	—
I	20	35.0 (21–42)	17
	30	31.5 (24–37)	5
II	200	35.0 (27–44)	17
	300	33.0 (28–53)	10
III	30	32.5 (29–39)	8
	50	23.0 (19–33)	–23
IV	30	34.0 (28–59)	13
	50	31.5 (28–37)	5
VI	200	30.0 (22–53)	0
	300	38.0 (22–51)	27
VII	200	30.0 (21–37)	0
IX	100	31.0 (27–47)	3
	200	27.0 (21–45)	–10
Cyclophosphamide	20	31.5 (26–38)	5
	30	40.0 (16–62)	33

*Drugs were administered orally 3 times a wk for 4 wks beginning on Day 1 after tumor implantation.

tively, while the optimal dose of the 1-methyl and 1-ethyl derivatives was 50 mg/kg/day. Higher doses of the former two derivatives produced the same degree of toxicity as lower doses of the latter two.

On the other hand, the 1-phenethyl derivative was not as effective in mice bearing B16 melanoma (table 3). In fact, the lifespan of mice inoculated sc with the melanoma was slightly affected by only two of the compounds tested: 1-hexyl carbamoyl-5-fluorouracil, which increased the lifespan 27% at the optimal dose, and cyclophosphamide, which increased the lifespan 33% at the optimal dose. The other six compounds tested orally against the sc B16 melanoma were inactive at the doses tested.

Effect of 1-Hexyl and 1-Phenethyl Carbamoyl-5-fluorouracil on Advanced Lewis Lung Carcinoma and Influence of the Schedule of Administration

In order to compare the effect of the two active compounds (1-hexyl and 1-phenethyl carbamoyl-5-fluorouracil), activity against advanced Lewis lung carcinoma was examined. Results of treatment with the compounds are shown in table 4 and figure 1. Using a schedule of three doses per week, the 1-hexyl derivative was more effective than the 1-phenethyl derivative, which prolonged the median lifespan only 10% longer than that of the control group. Lung metastases were slightly inhibited at optimal doses.

Daily treatment with the 1-hexyl and 1-phenethyl derivatives was more effective than intermittent treatment. Mice receiving a 150-mg/kg/day dose of 1-hexyl carbamoyl-5-fluorouracil and a 100-mg/kg/day dose of 1-phenethyl carbamoyl-5-fluorouracil showed a significant ILS (44% and 58% respectively). The 1-phenethyl derivative completely inhibited the growth of tumor at the site of implantation (fig 1) and the growth of lung metastases (table 4) at optimal doses.

TABLE 4.—Effect of 1-hexyl (VI) and 1-phenethyl (IX) carbamoyl derivatives of 5-FU (delayed treatment) on survival and lung metastases of mice bearing sc Lewis lung carcinoma

Experiment No.	Compound No.	Dose (mg/kg/day)	Schedule*	Survival time (days)		ILS (%)	Lung metastases†
				Median	Range		
1	Control	—	—	29.0	22–36	—	6/6
	VI	100	3 times/wk	33.0	25–36	14	5/6
		200	3 times/wk	38.5	29–54	33	4/6
		300	3 times/wk	32.0	25–32	10	1/6
	IX	100	3 times/wk	32.0	21–40	10	4/6
		200	3 times/wk	29.5	25–44	2	2/6
	Cyclophosphamide	30	3 times/wk	37.0	31–57	28	2/6
2	Control	—	—	26.0	21–28	—	6/6
	VI	100	Daily	34.5	27–46	33	6/6
		150	Daily	37.5	29–52	44	6/6
		200	Daily	30.0	30–34	15	0/6
	IX	50	Daily	32.0	32–38	23	6/6
		100	Daily	41.0	31–60	58	0/6
		150	Daily	30.0	28–34	15	0/6
	Cyclophosphamide	10	Daily	28.5	26–40	10	6/6
	5-FU	20	Daily	29.0	19–40	12	6/6

*Drug was administered orally 3 times a wk in experiment 1 and daily in experiment 2, starting on Day 14 after tumor implantation until death.

†Values given are the No. of mice with metastases/No. of mice tested.

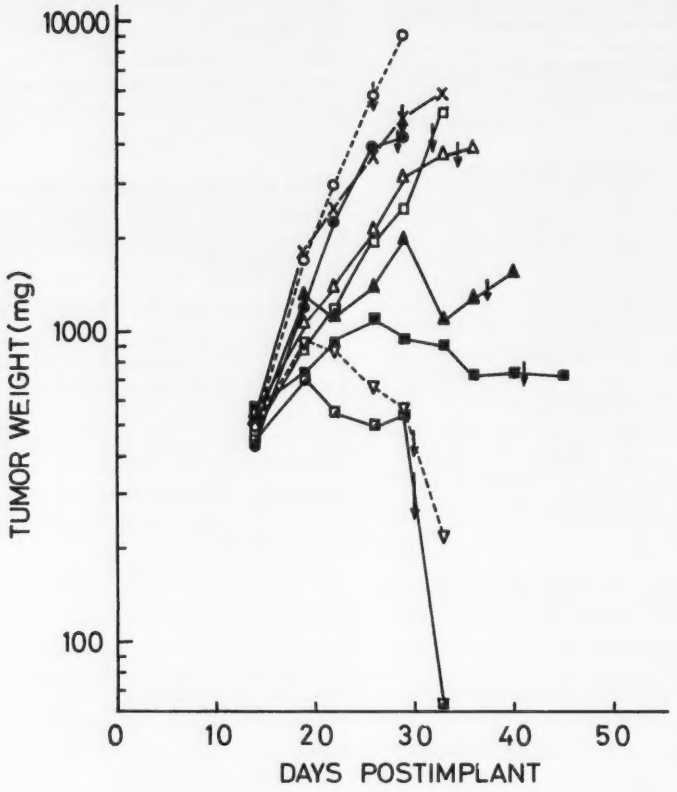


FIGURE 1.—Growth rate of advanced Lewis lung carcinoma treated with 5-FU, cyclophosphamide, 1-hexyl carbamoyl-5-fluorouracil, and 1-phenethyl carbamoyl-5-fluorouracil by daily administration. Arrows indicate the median day of death. ---○--- = control (experiment 2); —— × —— = 5-FU (20 mg/kg/day); ——●—— = cyclophosphamide (10 mg/kg/day); ——△—— = 1-hexyl derivative (100 mg/kg/day); ——▲—— = 1-hexyl derivative (150 mg/kg/day); ---▽--- = 1-hexyl derivative (200 mg/kg/day); ——□—— = 1-phenethyl derivative (50 mg/kg/day); ——■—— = 1-phenethyl derivative (100 mg/kg/day); ——◪—— = 1-phenethyl derivative (150 mg/kg/day).

DISCUSSION

The Lewis lung carcinoma test system has been used as a syngeneic slow-growing tumor model at the National Cancer Institute, Bethesda, MD, and it is resistant to most established antitumor agents (4). Cyclophosphamide was the most effective among the agents tested against the tumor (4). Early ip treatment with cyclophosphamide of mice implanted sc with the tumor yielded tumor-free mice, but delayed ip treatment only inhibited tumor growth for a short time (6). 5-FU, the most effective of the established antimetabolites in this tumor system, resulted in a lesser inhibition of tumor growth with no prolongation of median survival time by delayed ip treatment (6). This model is useful for examining the effects of an agent on tumor growth using long-term oral administration, as well as the effect on lung metastases of the tumor. With long-term administration, 5-FU was slightly active against Lewis lung carcinoma but FT was markedly active using oral administration (7). The 1-alkyl carbamoyl derivatives of 5-FU were as active as FT against the tumor (table 2) and two derivatives (1-hexyl and 1-phenethyl) were also active against the advanced tumor (table 4).

The 1-methyl and 1-ethyl carbamoyl derivatives of 5-FU were not as effective in this tumor, although they were markedly active against rapidly growing tumors (3). The 1-hexyl and 1-phenethyl derivatives were more

effective using daily administrations. On the other hand, 5-FU derivatives were almost inactive against B16 melanoma using long-term administration. The 1-hexyl derivative is found to be the most active compound among the derivatives of 5-FU in rapidly (3) and slow-growing tumor systems, and a phase I study of this agent is now in progress at the National Cancer Center Hospital, Tokyo, Japan. However, the 1-phenethyl derivative was the most active against advanced Lewis lung carcinoma using daily administration and completely inhibited lung metastases. It is more effective than cyclophosphamide, which was used as a positive control, and the 1-hexyl derivative. Further evaluation of the 1-phenethyl derivative should be carried out by additional trials in other metastases models.

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Alterations in the Toxicity and Antitumor Activity of Methyl-CCNU in Mice Following Pretreatment With Either Phenobarbital or SKF 525A [1,2]

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SUMMARY

We examined the effects of pretreatment with either SKF 525A or phenobarbital on the toxicity and antitumor action of methyl-CCNU (MeCCNU) in B6D2F$_1$ mice. Pretreatment with SKF 525A (50 mg/kg ip) 30 minutes before a single ip injection of MeCCNU (31.5 or 42.0 mg/kg) increased the lethal toxicity of MeCCNU in normal mice. In contrast, pretreatment with phenobarbital (100 mg/kg ip, daily for 4 days) followed on Day 5 by MeCCNU (63 mg/kg) decreased the lethal toxicity of MeCCNU. The nadir in bone marrow nucleated cell numbers on Day 3 after MeCCNU (21 mg/kg) was further depressed by pretreatment with SKF 525A, whereas phenobarbital pretreatment had a sparing effect on marrow cell numbers. Pretreatment with prochlorperazine (80 mg/kg ip), an antiemetic frequently used with antineoplastic agents, increased the lethal toxicity of MeCCNU (42 mg/kg). Mice were given an sc injection of 1×10^6 L1210 ascites tumor cells on Day 0. They were then pretreated with either phenobarbital (100 mg/kg ip, Days 2, 3, 4, and 5) or SKF 525A (50 mg/kg ip, Day 6). MeCCNU (10.5 mg/kg ip) was then administered on Day 6. MeCCNU alone resulted in an increase in median lifespan of 252% with two long-term survivors among ten mice whereas phenobarbital pretreatment resulted in an increase in median lifespan of only 47% with no survivors among ten mice. Pretreatment with SKF 525A resulted in an increase in median lifespan of 380% and five long-term survivors among ten mice, but this was not significant as compared to MeCCNU alone. At a higher dose of MeCCNU (21.0 mg/kg), neither phenobarbital nor SKF 525A pretreatment altered the increase in median lifespan or number of long-term survivors. The data indicate that concurrent treatment with agents which modify liver microsomal enzyme activities can affect the toxicity and antitumor action of MeCCNU.

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Methyl-CCNU (MeCCNU) is a lipid-soluble antitumor agent with activity against a wide variety of animal and human neoplasms (1). MeCCNU as well as other clinically useful nitrosoureas including CCNU and BCNU undergo spontaneous decomposition under physiologic conditions with liberation of potential alkylating moieties (2,3). In addition to their spontaneous decomposition, the nitrosoureas are metabolized by liver microsomal enzymes, and hydroxylated metabolites of MeCCNU and CCNU, which have antitumor activity, have been identified (4–8). Various studies indicate that the rate of the cytochrome P-450-dependent hydroxylation of MeCCNU and CCNU exceeds their rates of spontaneous decomposition. It has been suggested that the antitumor activity of these nitrosoureas may be mediated to a considerable degree by their hydroxylated metabolites (9). For example, the *cis*-4-hydroxy derivative of CCNU is its major metabolite and accounts for > 45% of administered CCNU after phenobarbital pretreatment of rats (6). Furthermore, phenobarbital pretreatment of animals resulted in an in-

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creased rate of formation of hydroxylated metabolites of CCNU and MeCCNU as measured both in vitro and in vivo (4,6–8,10). With respect to clinical studies, it was found that, after a 1-hour iv infusion of CCNU to patients, approximately 75% of plasma drug concentration was hydroxylated metabolites (11).

It seemed to be of theoretic as well as practical importance to determine if pretreatment with agents which affect the activity of liver microsomal enzymes could influence the toxicity and antitumor action of MeCCNU. Therefore, we examined the effect of either phenobarbital or SKF 525A pretreatment on the action of MeCCNU in normal mice and mice bearing a solid L1210 tumor.

MATERIALS AND METHODS

MeCCNU was obtained as a gift from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. SKF 525A and prochlorperazine edisylate were obtained as gifts from Smith, Kline and French Laboratories, Philadelphia, PA. Sodium phenobarbital was obtained from Merck and Co, Rahway, NJ.

Animals and Tumors

Male (C57BL/6 × DBA/2)F_1 (hereafter called B6D2F_1) mice, 18–22 g, and male DBA/2 mice, 18–20 g, were obtained from Sprague-Dawley Laboratories, Madison, WI. Animals were caged in an air-conditioned room lighted from 8 AM to 8 PM and had free access to a standard Purina laboratory chow diet and tap water. The L1210 tumor in the ascites form, obtained from Hazelton Laboratories, Falls Church, VA, was maintained in male DBA/2 mice.

Chemotherapeutic Agents

MeCCNU was dissolved in 0.5 ml of 95% ethanol containing 0.05 ml of Tween 80, and then the drug was made up to a final volume of 10 ml with 0.9% NaCl. The resulting suspension was maintained by stirring. The final concentration of the drug permitted injection of 0.01 ml/g of mouse body weight. Injections were completed within 15 minutes after drug dispersion. Prior to treatment, animals were weighed individually.

Sodium phenobarbital, SKF 525A, or prochlorperazine edisylate was dissolved just prior to use in 0.9% NaCl and the final concentrations of the drug permitted injections of 0.01 mg/g of mouse body weight.

Administration Schedule for Chemotherapeutic Agents

For normal non-tumor-bearing mice, liver microsomal enzyme activities were induced by administration of four successive daily single ip injections of sodium phenobarbital (100 mg/kg). A single ip injection of MeCCNU was then administered 24 hours after the last dose of phenobarbital. In order to inhibit liver microsomal enzymes, a single ip injection of SKF 525A (50 mg/kg) was administered 30 minutes before a single ip injection of the nitrosourea. A single ip injection of prochlorperazine edisylate (80 mg/kg) was administered 30 minutes before a single ip injection of MeCCNU. Animals were observed for individual days of death and number of 30-day survivors after treatment with the nitrosourea.

For studies in tumor-bearing mice, the animals were given sc injections of 1×10^6 L1210 ascites cells into the right axilla on Day 0. Animals were then randomly divided into groups of ten each. Sodium phenobarbital (100 mg/kg ip) was administered on Days 2, 3, 4, and 5, and MeCCNU was administered as a single ip injection on Day 6. On Day 6 after tumor inoculation, SKF 525A was administered as a single ip injection (50 mg/kg) 30 minutes before a single ip injection of MeCCNU. Animals were observed for individual days of death and long-term survivors (> 51 days after tumor implantation, which corresponded to > 45 days after MeCCNU treatment). The increase in median lifespan of the treatment groups as compared to the control groups was determined as previously described (12).

Effect of Chemotherapeutic Agents on Bone Marrow Nucleated Cellularity

Normal non-tumor-bearing mice, in groups of four each, were pretreated with either sodium phenobarbital or SKF 525A and then given a single ip injection of MeCCNU. On Day 3 after MeCCNU, the number of nucleated cells per femur was determined by the method of Fruham (13). Preliminary experiments showed that the nadir of bone marrow nucleated cellularity occurred on Day 3 after MeCCNU treatment.

RESULTS

Effect of Pretreatment With SKF 525A on the Lethal Toxicity of MeCCNU

Table 1 shows the effects of pretreatment with SKF 525A, an inhibitor of liver microsomal enzymes, on the lethal toxicity of varying doses of MeCCNU. A single ip injection to male B6D2F_1 mice of 21.0, 31.5, 42.0, or 63.0 mg/kg of MeCCNU is reported to be 0.5, 0.75, 1.0, and 1.5 times, respectively, the LD10 dose (14). As can be seen, SKF 525A followed by MeCCNU (31.5 mg/kg) increased the lethality of MeCCNU in that there was only one 30-day survivor among ten animals as compared to nine of ten with MeCCNU (31 mg/kg) alone. Similarly, SKF 525A followed by MeCCNU (42.0 mg/kg) resulted in no 30-day survivors among ten animals as compared

TABLE 1.—Effect of pretreatment with SKF 525A on the lethal toxicity of MeCCNU in B6D2F$_1$ mice

Treatment	Dose of MeCCNU (mg/kg)	Individual days of death	30-day survivors/ total treated*
SKF 525A	—	—	10/10
MeCCNU	21.0	—	10/10
SKF 525A → MeCCNU	21.0	14,15	8/10
MeCCNU	31.5	10	9/10
SKF 525A → MeCCNU	31.5	6,6,6,8,9,9,13,13,16	1/10†
MeCCNU	42.0	11	9/10
SKF 525A → MeCCNU	42.0	2,5,5,5,5,6.9,11,12,13	0/10‡

*Groups of 10 mice each were treated with a single ip injection of either SKF 525A (50 mg/kg) alone, SKF 525A (50 mg/kg) 30 mins before MeCCNU, or MeCCNU alone. Survivors were determined daily for the next 30 days.

†$P < 0.005$ compared to MeCCNU (31.5 mg/kg) alone by chi-square analysis.

‡$P < 0.005$ compared to MeCCNU (42 mg/kg) alone by chi-square analysis.

to nine of ten with MeCCNU (42.0 mg/kg) alone. At a lower dose of MeCCNU (21.0 mg/kg), pretreatment with SKF 525A failed to markedly increase its lethality. When the experiment was repeated (not shown) similar results were obtained.

Effect of Phenobarbital Pretreatment on the Lethal Toxicity of MeCCNU

Table 2 shows the effects of pretreatment with phenobarbital, an inducer of liver microsomal enzymes, on the lethal toxicity of MeCCNU in B6D2F$_1$ mice. As can be seen, pretreatment with phenobarbital decreased the lethal toxicity of MeCCNU (63.0 mg/kg), resulting in seven 30-day survivors among ten animals as compared to no 30-day survivors among ten animals after MeCCNU (63.0 mg/kg) alone. However, the protective effect of phenobarbital pretreatment was not seen with a higher dose of MeCCNU (84.0 mg/kg). When the experiment was repeated (not shown) similar results were obtained.

Effect of Pretreatment With Either SKF 525A or Phenobarbital on Bone Marrow Nucleated Cellularity

One of the major dose-limiting toxic effects of MeCCNU in clinical use is bone marrow suppression (15). Therefore, we attempted to see if the alterations in toxicity from MeCCNU after pretreatment with either SKF 525A or phenobarbital correlated in a quantitative manner with the effects on bone marrow nucleated cellularity. As can be seen (table 3), SKF 525A pretreatment followed by MeCCNU (21.0 mg/kg) caused a greater decrease in bone marrow nucleated cell numbers (16% of control) than did MeCCNU (21.0 mg/kg) alone (31% of control). In fact, the decrease in the number of bone marrow nucleated cells due to SKF 525A followed by MeCCNU (21.0 mg/kg) was comparable to that produced by MeCCNU (42.0 mg/kg) alone (11% and 16% of control respectively).

In contrast, phenobarbital pretreatment followed by

TABLE 2.—Effects of pretreatment with phenobarbital on the lethal toxicity of MeCCNU in B6D2F$_1$ mice

Treatment	Dose of MeCCNU (mg/kg)	Individual days of death	30-day survivors/ total treated*
Phenobarbital	—	—	10/10
MeCCNU	42	15	9/10
Phenobarbital → MeCCNU	42	—	10/10
MeCCNU	63	5,5,5,6,6,6,6,7,10,14	0/10
Phenobarbital → MeCCNU	63	5,6,13	7/10†
MeCCNU	84	4,4,4,5,5,5,5,5,5,7	0/10
Phenobarbital → MeCCNU	84	5,5,5,5,6,6,6,6,7,7	0/10

*Groups of 10 mice each were treated with a single ip injection of phenobarbital (50 mg/kg) daily for 4 days. 24 hrs after the last injection of phenobarbital, a single ip injection of MeCCNU was administered. Survivors were determined daily for the next 30 days.

†$P < 0.05$ compared to MeCCNU (63 mg/kg) alone by chi-square analysis.

TABLE 3.—Effect of pretreatment with either SKF 525A or phenobarbital on the MeCCNU-induced depression of the Nos. of nucleated cells per femur of $B6D2F_1$ mice

Experiment No.	Treatment	Dose of MeCCNU (mg/kg, ip)	Nucleated cells per femur × 10^{-6} ± SE	% control*
1	Control	—	17.7 ± 0.58	—
	SKF 525A	—	18.3 ± 0.80	103
	MeCCNU	21	5.4 ± 0.82	31
	MeCCNU	42	1.99 ± 0.36	11
	SKF 525A → MeCCNU	21	2.88 ± 0.36†	16†
2	Control	—	19.6 ± 0.85	—
	Phenobarbital	—	17.2 ± 1.3	88
	MeCCNU	21	5.18 ± 0.45	26
	Phenobarbital → MeCCNU	21	9.14 ± 0.68‡	47‡

*For experiment 1 groups of 4 mice each were treated with a single ip injection of either SKF 525A (50 mg/kg) alone, SKF 525A (50 mg/kg) 30 mins before MeCCNU, or MeCCNU alone. For experiment 2 groups of 4 mice each were treated with a single ip injection of phenobarbital (100 mg/kg) daily for 4 days, and then on Day 5 they were given a single ip injection of MeCCNU. For both experiments 1 and 2 at 72 hrs after administration of MeCCNU animals were killed and the No. of nucleated cells per femur was determined as described in the Materials and Methods section. The values obtained for the paired femora of each mouse were averaged, and the results were expressed as the mean ± SE of 4 mice per treatment group.

†$P < 0.05$ compared to MeCCNU (21 mg/kg) alone by Student's t test analysis.

‡$P < 0.005$ compared to MeCCNU (21 mg/kg) alone by Student's t test analysis.

TABLE 4.—Effect of pretreatment with prochlorperazine edisylate on the lethal toxicity of MeCCNU in $B6D2F_1$ mice

Treatment	Individual days of death	30-day survivors/total treated*
Prochlorperazine edisylate	—	10/10
MeCCNU	5,6	8/10
Prochlorperazine edisylate → MeCCNU	4,6,6,7,7,8,9,13	2/10†,‡

*Groups of 10 mice each were treated with a single ip injection of either prochlorperazine edisylate (80 mg/kg) alone, prochlorperazine edisylate (80 mg/kg) 30 mins before MeCCNU (42 mg/kg), or MeCCNU (42 mg/kg) alone. Survivors were determined daily for the next 30 days.

†$P < 0.05$ compared to MeCCNU (42 mg/kg) alone by chi-square analysis.

‡$P < 0.005$ compared to prochlorperazine edisylate alone by chi-square analysis.

MeCCNU (21.0 mg/kg) had a sparing effect on bone marrow nucleated cell numbers as compared to MeCCNU (21.0 mg/kg) alone (47% and 26% of control respectively). Thus, increased or decreased lethal toxicity of MeCCNU due to pretreatment with either SKF 525A or phenobarbital respectively (tables 1 and 2) correlated with the extent of depression of bone marrow nucleated cellularity at a dose of MeCCNU of 21 mg/kg.

Effect of Prochlorperazine Pretreatment on MeCCNU Toxicity

Acute gastrointestinal distress characterized by nausea and vomiting is often seen following clinical use of MeCCNU (16). Prophylactic measures to minimize this side effect include pretreatment with phenothiazine antiemetics such as prochlorperazine. It was possible that competition between prochlorperazine and MeCCNU for microsomal enzyme metabolism might increase the lethal toxicity of MeCCNU in mice. As can be seen (table 4), prochlorperazine administered 30 minutes before MeCCNU (42 mg/kg) increased the lethal toxicity of MeCCNU in that there were only two 30-day survivors among ten animals as compared to eight of ten with MeCCNU alone.

Effect of Pretreatment With Either Phenobarbital or SKF 525A on the Antitumor Activity of MeCCNU

Increased or decreased toxicity of MeCCNU was seen after pretreatment of normal mice with either SKF 525A or phenobarbital respectively. Therefore, it was important to determine if these two agents could alter the antitumor activity of MeCCNU. As can be seen (table 5), the increase in median survival time of 252% with MeCCNU (10.5 mg/kg) alone was reduced to 47% following phenobarbital pretreatment. Pretreatment with SKF

TABLE 5.—Effect of pretreatment with either phenobarbital or SKF 525A followed by treatment with MeCCNU on the survival times of $B6D2F_1$ mice bearing solid L1210 tumor

Experiment No.	Treatment	Dose of MeCCNU (mg/kg)	Median survival time in days (range)	% increase in median survival time	51-day survivors/total treated*
1	Saline	—	9.5 (8–11)	—	0/10
	Phenobarbital	—	9.0 (8–10)	—	0/10
	SKF 525A	—	9.0 (8–10)	—	0/10
	MeCCNU	10.5	33.5 (17–> 51)	252†	2/10
	Phenobarbital → MeCCNU	10.5	14.0 (11–14)	47‡	0/10
	SKF 525A → MeCCNU	10.5	45.5 (20–> 51)	380§	5/10
2	Saline	—	9.0 (7–10)	—	
	Phenobarbital	—	9.0 (8–10)	—	
	SKF 525A	—	9.0 (8–10)	—	
	MeCCNU	21	51.0 (22–> 51)	467¶	8/10
	Phenobarbital → MeCCNU	21	51.0 (23–> 51)	467**	7/10
	SKF 525A → MeCCNU	21	51.0 (20–> 51)	467**	9/10

*Groups of 10 mice each were given sc injections of 1×10^6 L1210 ascites cells into the right axilla on Day 0. Phenobarbital (100 mg/kg, ip) was administered as a single daily ip injection on Days 2, 3, 4, and 5 followed on Day 6 by a single ip injection of MeCCNU (10.5 or 21 mg/kg as indicated). A single ip injection of SKF 525A (50 mg/kg) was administered on Day 6 after tumor inoculation 30 mins before a single ip injection of MeCCNU (10.5 or 21 mg/kg as indicated). Animals in both the treatment and control groups were observed for day of death and median survival times determined as described in the Materials and Methods section.

†$P < 0.0005$ compared to saline control by chi-square analysis.

‡$P < 0.0005$ compared to MeCCNU (10.5 mg/kg) alone by chi-square analysis.

§Not significant compared to MeCCNU (10.5 mg/kg) alone by chi-square analysis.

¶$P < 0.0005$ compared to saline control by chi-square analysis.

**Not significant compared to MeCCNU (21 mg/kg) alone by chi-square analysis.

525A as compared to MeCCNU alone showed a trend toward increasing the median survival time to 380% as well as the number of long-term survivors; however, the results were not statistically significant. On the other hand, using a higher dose of MeCCNU (21 mg/kg), pretreatment with either phenobarbital or SKF 525A did not change the response when compared to MeCCNU alone. When the experiment was repeated (not shown) similar results were obtained.

DISCUSSION

The rate of microsomal metabolism of MeCCNU is considered to be sufficiently high to allow enzymatic transformation of large portions of an administered dose of MeCCNU as compared to chemical decomposition (5). Using the $B6D2F_1$ mouse, which is considered to be a quantitative and partially qualitative predictor of MeCCNU toxicity in man (14), we found that pretreatment with phenobarbital, which should increase the rate of microsomal metabolism of MeCCNU, decreased the biologic activity of MeCCNU whereas the converse was true after pretreatment with SKF 525A. Our data are consistent with the concept that a substantial enough portion of a therapeutic dose of MeCCNU may be metabolized by liver microsomal enzymes so that pretreatment with either SKF 525A or phenobarbital, both of which modify the activities of these enzymes, can alter the toxicity and antitumor action of MeCCNU.

Patients undergoing cancer chemotherapy are routinely treated with a variety of drugs, including anticancer agents, which can modify the activities of liver microsomal agents (17). We found that pretreatment with SKF 525A enhanced the bone marrow suppressive effect of MeCCNU whereas phenobarbital pretreatment had a sparing effect on bone marrow suppression due to MeCCNU. These effects on bone marrow nucleated cellularity were consistent with our observation that pretreatment with either SKF 525A or phenobarbital increased and decreased, respectively, the lethal toxicity of MeCCNU. These data indicate that the clinical toxicity of MeCCNU may be altered inadvertently in patients who are concurrently treated with other drugs which modify liver microsomal enzyme activity.

We demonstrated such an interaction in our model by showing that pretreatment of mice with the phenothiazine antiemetic agent prochlorperazine increased the lethal toxicity of MeCCNU. Presumably, the increased toxicity of MeCCNU was due to a decrease in its rate of microsomal cytochrome P-450 metabolism in the presence of prochlorperazine, which is also metabolized by the same enzyme system (18). Competition between two concurrently administered drugs for microsomal enzyme metabolism, with a resultant increase in the action and/or toxicity of one of the drugs, has been frequently described (19). The possibility cannot be excluded that the increase in the lethality of MeCCNU after prochlorperazine pretreatment may be due to mechanisms other than

inhibition by prochlorperazine of MeCCNU metabolism. For example, phenothiazines have a variety of actions, including alteration in membrane permeability, binding to plasma protein, and effects on various enzyme systems, which can account for, or contribute to, an adverse drug interaction when they are administered concurrently with another drug (18,20). Similarly, our experiments cannot exclude the possibility that modifications in the toxicity of MeCCNU by pretreatment with either phenobarbital or SKF 525A may be due to mechanisms other than altered biotransformation of MeCCNU.

Of further practical concern are the effects of either phenobarbital or SKF 525A pretreatment on the antitumor activity of MeCCNU. Our data indicate that neither prior induction nor inhibition of liver microsomal enzymes is likely to afford any therapeutic advantage with MeCCNU. This is because a decrease in the toxicity of MeCCNU due to phenobarbital pretreatment also decreased the antitumor activity of MeCCNU (10.5 mg/kg) against the solid mouse L1210 tumor (table 5). At a higher dose of MeCCNU (21 mg/kg), phenobarbital pretreatment had no apparent effect on the antitumor action of MeCCNU. Presumably, the increased rate of microsomal metabolism of MeCCNU after phenobarbital pretreatment did not decrease the activity of a substantial enough portion of the higher dose of MeCCNU to cause any modification in its antitumor effect. Thus, for the L1210 tumor, which is highly responsive to MeCCNU (21), phenobarbital pretreatment did not diminish the antitumor action of MeCCNU at all doses within the therapeutic range. However, for other rodent tumors, or human tumors which may be less responsive to MeCCNU, an increase in liver microsomal enzyme metabolism of MeCCNU might markedly decrease the antitumor effect of MeCCNU over a wider range of doses.

On the other hand, pretreatment with SKF 525A showed a tendency toward increasing the antitumor action of MeCCNU (10.5 mg/kg) against mice bearing a solid L1210 tumor (table 5). However, pretreatment with SKF 525A produced no apparent increase in the antitumor action of MeCCNU (21 mg/kg) as compared to MeCCNU alone. Nevertheless, since SKF 525A increases the toxicity of MeCCNU, any tendency toward increasing the antitumor action of MeCCNU may also increase host toxicity (tables 1 and 3).

Pretreatment of rats or mice with phenobarbital leads to an increased rate of hydroxylation of MeCCNU and CCNU as well as a change in the relative amounts of hydroxylated metabolites formed from liver microsomal enzymes in vitro (6,8,10). Hansch et al (22) predicted that metabolic transformation of CCNU to a more hydrophilic nitrosourea (eg, ring-hydroxylated metabolites of CCNU) should yield a more potent and less toxic antitumor agent. Various hydroxylated metabolites of CCNU have been prepared and their toxicity and antitumor activity assessed (23,24). Ring-hydroxylated metabolites of CCNU differ from the parent compound with respect to plasma half-lives, distribution coefficients, and alkylating and carbamoylating activity (23,24). Whether any hydroxylated metabolites of MeCCNU have any therapeutic advantage over MeCCNU remains to be determined (10).

In addition to the microsomal hydroxylation of MeCCNU, a cytochrome P-450-dependent denitrosation is also an important pathway in its metabolism. Furthermore, phenobarbital pretreatment of mice alters the relative rates of formation by liver microsomes of hydroxylated metabolites of MeCCNU and its denitrosated metabolite, 1-(2-chloroethyl)-3-(*trans*-4-methylcyclohexyl)urea (8). The extent to which pretreatment with either phenobarbital or SKF 525A may modify the relative rates of hydroxylation as compared to denitrosation of MeCCNU in vivo and thereby contribute to the altered toxicity of MeCCNU remains to be determined.

Levin et al (25) examined the effects of repeated oral administration of phenobarbital on the antitumor activity of BCNU, CCNU, and 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperdyl)-1-nitrosourea (PCNU) against the intracerebral 9L tumor in rats. They showed that phenobarbital pretreatment eliminated the antitumor activity of BCNU and reduced the activity of CCNU and PCNU. Phenobarbital pretreatment also reduced the systemic toxicity of BCNU. The in vitro rate of BCNU disappearance in the 9000 × *g* supernatants and microsomes from the livers of phenobarbital-pretreated rats was 5 times faster than the rate of disappearance of BCNU in supernatants from normal rats. The authors concluded that repeated oral administration of phenobarbital induces a change in liver microsomal enzymes which increases the clearance of BCNU, thereby reducing the antitumor activity of BCNU and other nitrosoureas.

It is possible that patients treated with drugs which either induce or inhibit liver microsomal enzymes would have an altered capability of metabolizing MeCCNU. Alterations in both the rate of metabolism and relative formation of individual metabolites of MeCCNU could modify its toxicity and antitumor action. Our data indicate that it might be worthwhile to assess the possible effect of concurrent treatment with other drugs on the toxicity and antitumor effectiveness of MeCCNU in cancer patients.

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Phase I Study of *N*-(Phosphonacetyl)-L-aspartic Acid (PALA)[1,2]

John S. Kovach,* Allan J. Schutt, Charles G. Moertel, and Michael J. O'Connell[3]

SUMMARY

N-(Phosphonacetyl)-L-aspartic acid, an inhibitor of aspartate transcarbamylase, was administered to 25 patients with advanced cancer by 10-minute infusion daily × 5 consecutive days to determine the toxicity and to look for evidence of therapeutic effect. Planned dose escalations ranged from 100 to 1250 mg/m² (daily dose). Nausea, vomiting, and diarrhea were the most frequent toxic effects, with three of six patients treated at a daily dose of 1250 mg/m² having severe diarrhea. Other toxic effects were encountered rarely and were not dose-limiting; these included mild leukopenia, thrombocytopenia, rash, stomatitis, and increases in SGOT. One patient with a widely metastatic carcinoid of unknown origin had an objective response lasting 6 weeks.

[Cancer Treat Rep 63:1909–1912, 1979]

N-(Phosphonacetyl)-L-aspartic acid (PALA) was synthesized by Collins and Stark (1) and others as a transition-state inhibitor of aspartate transcarbamylase (2–4), an enzyme involved in de novo biosynthesis of pyrimidine nucleotides. PALA has significant activity against B16 melanoma, sc implanted Lewis lung carcinoma, and a number of other solid tumors in rodents (3,5). Johnson et al (6) demonstrated that PALA does not impair rejection of a tumor allograft system in mice and that, even at doses which are greater than the LD50 in $B6D2F_1$ mice, rapidly proliferating tissue such as the bone marrow and epithelial lining of the small intestine are spared. Sensitivity or resistance to PALA in vivo and in vitro depends upon intracellular concentrations of aspartate transcarbamylase (6).

In dogs, the major toxic effects of PALA given iv were weight loss, adipsia, anorexia, diarrhea, emesis, hematemesis, rectal bleeding, sedation, anesthesia, seizures, hyperactivity, dyspnea, ataxia, incontinence, and salivation (7). In monkeys, the major toxic effects were diarrhea, skin flaking or peeling, alopecia, sedation, incontinence, dyspnea, and ataxia. Both species showed increases in serum creatinine, creatine phosphokinase, serum LDH, BUN, and prothrombin time, and decreases in serum electrolytes. No electroencephalographic abnormalities were found. The drug was not felt to be toxic to the hemopoietic system, although some monkeys showed transient decreases in their total wbc count. There was a suggestion that toxicity was schedule-dependent since repeated doses were more toxic than intermittent doses.

The highest nontoxic dose in the dog, the more sensitive of the two species, was 1200 mg/m² with the 1-day schedule and 300 mg/m² with the 5-day schedule.[4] The toxic dose low in the dog with the 5-day schedule has not been reported.

PALA was as effective on an intermittent schedule as it was on a daily schedule against P388 or L1210 leukemia (5), but was found to produce a higher percentage of cures against the Lewis lung carcinoma when administered on Days 1–4 after tumor implantation (6). For this reason, and because of its apparent lack of hematologic toxicity, PALA was administered on a daily × 5 day schedule. The starting dose was one third of the highest nontoxic dose on the 5-day schedule in the dog: 100 mg/m²/day × 5 days.[4]

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[4]Clinical brochure: PALA (NSC-224131). Prepared by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, Dec 1977, 45 pp.

PATIENTS AND METHODS

All patients had histologic or cytologic confirmation of unresectable cancer for which no other more conventional means of therapy would offer reasonable hope of cure or significant palliation. All patients were fully informed of the precise diagnosis and nature of their disease and signed an informed consent form. Patients were excluded from the study according to the following criteria: a wbc count of $< 4100/\mu l$, a platelet count of $< 130{,}000/\mu l$, a hemoglobin level of < 10 g/dl, any elevation of direct-reacting serum bilirubin, a serum creatinine level of > 1.4 mg/dl, or a performance status of 4 according to the Eastern Cooperative Oncology Group scale. Patients were also excluded if they had major surgery during the previous 30 days, radiation therapy to $> 15\%$ of the bone marrow within 30 days, radiation therapy to $> 30\%$ of the bone marrow at any time, therapy with cytotoxic agents within 21 days (42 days for nitrosoureas), active infection, frequent vomiting, severe anorexia, or a psychologic state which precluded obtaining informed consent.

Within 3 days of initiating treatment, all patients had a complete medical history and physical examination and the following laboratory measurements: wbc and differential counts, hemoglobin, platelet count, urinalysis, serum creatinine, SGOT, alkaline phosphatase, direct and total bilirubin, electroencephalogram, electrocardiogram, serum amylase, and prothrombin time. Weight, symptomatic status, and performance status were assessed on the day of initiation of therapy. All parameters, as well as a complete physical examination, were repeated at 3-week intervals. Hematologic and blood chemistry studies were repeated on Days 3 and 6 counting from the first day of treatment. Wbc and platelet counts and hemoglobin levels were obtained twice weekly and serum creatinine, SGOT, and prothrombin time were obtained once weekly as long as the patient remained in the study. Drug courses were repeated at 6-week intervals. Patients were entered in groups of three and observed for 3 weeks before new patients were entered at a higher dose level. Patients who had no toxic effects and remained stable or showed improvement over a 6-week period of observation were re-treated at the next planned dose level.

The study population consisted of 25 patients, 15 females and ten males. The median age was 55 years, with a range of 21–66 years. The mean performance status was 1.2 (Eastern Cooperative Oncology Group scale, 0–4). Twenty-three patients had received prior chemotherapy, one had received prior radiotherapy only, one had received no prior therapy, and seven had received prior radiotherapy and chemotherapy.

PALA (supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD) was administered as a sterile aqueous solution at a concentration of 100 mg/ml and was infused over a 10-minute period daily $\times$ 5 consecutive days.

RESULTS

Twenty-five patients received a total of 39 courses of PALA. The daily dose levels studied were 100, 350, 600, 800, 1000, and 1250 mg/m^2/day $\times$ 5 consecutive days. Toxic effects following all courses of therapy are summarized in table 1. Nausea and vomiting occurred in approximately 50% of patients at all dose levels. This reaction was confined to days of treatment and was ameliorated by antiemetics. Only three of 27 courses at doses of ≤ 1000 mg/m^2 were followed by moderate diarrhea. However, three of six patients treated at a dose of 1250 mg/m^2/day $\times$ 5 consecutive days had severe diarrhea. In one patient severe diarrhea began on the fourth treatment day and the fifth treatment was not given. The diarrhea subsided over a 3-day period. Two other patients developed persistent watery diarrhea without bleeding starting on the sixth or seventh day counting from the first day of treatment. One patient was hospitalized promptly and recovered uneventfully with fluid replacement. The other patient, with an adenocarcinoma of the uterus widely metastatic to the abdomen and significant ascites, did not seek medical help until the fourth day of diarrhea and died from shock. Neither patient had significant impairment of hepatic or renal function, but both had significant ascites secondary to peritoneal metastases. Three patients tolerated successive courses of treatment at daily doses of 800, 1250, and 1250 mg/m^2; 1000, 1250, and 1250 mg/m^2; and 1250, 1250, and 1250 mg/m^2 respectively without experiencing gastrointestinal toxic effects.

Other toxic effects of PALA have not been dose-limiting. Only seven of 39 courses were associated with reversible leukopenia or thrombocytopenia. Leukopenia was noted after six courses at doses ranging from 600 to 1000 mg/m^2/day $\times$ 5 days. The lowest wbc count was $3000/\mu l$. There were two instances of thrombocytopenia, both occurring in patients who had previously received extensive chemotherapy. The nadir of thrombocytopenia was $67{,}000$ platelets/μl on the 27th day in one patient and $89{,}000$ platelets/μl on the 42nd day in the other patient after initial courses at 800 and 1250 mg/m^2 respectively. The latter patient had two additional courses at a daily dose of 1250 mg/m^2 followed by nadirs of thrombocytopenia of $74{,}000$ and $69{,}000$ platelets/μl on Days 28 and 22 respectively. Only five of 37 courses were associated with what appeared to be drug-induced elevations in SGOT at daily doses ranging from 600 to 1250 mg/m^2. These changes were mild and reversible and not accompanied by alterations in serum alkaline phosphatase or bilirubin. There was no evidence of renal toxicity. Two patients reported mild oral stomatitis for 48 hours approximately

TABLE 1.—Toxic effects following 5-day courses of PALA

Daily dose (mg/m^2)	No. of patients/No. of courses	No. of courses with— Nausea and/or vomiting	Diarrhea	Stomatitis	Leukopenia (< 4100 cells/μl)	Thrombocytopenia (< 130,000 platelets/μl)	Rash
100	3/3	1	0	0	0	0	0
350	3/3	2	0	0	0	0	0
600	5/6	4	1	1	2 (3500, 4000)	0	1
800	8/9	7	2	0	3 (3900, 3900, and 3000)	1 (67,000)	0
1000	4/6	1	0	2	1 (3100)	0	1
1250	6/12	1	3*	0	1+ (3300)	3† (96,000, 98,000, and 78,000)	0

*Severe (3 patients); *see* text.
†1 patient had 3 successive courses 6 wks apart; leukopenia occurred during the 3rd course.

2 weeks after treatment at a daily dose of 1000 mg/m^2 and one patient experienced redness and slight ulceration of a colostomy stoma for 3 days starting 10 days after the first course of treatment at a dose of 600 mg/m^2. Two patients developed a macular, erythematous, nonpruritic rash over the upper trunk, neck, and arms starting approximately 2 weeks after treatment and lasting for about 3 days. Slight hyperpigmentation remained in the affected areas after resolution of the rash.

As noted earlier, patients in this study had an exceptionally good performance status. Tumors included colorectal carcinoma (six patients), pancreatic carcinoma (four), lung carcinoma (four), gastric carcinoma (three), adenocarcinoma of unknown origin (two), leiomyosarcoma of the uterus (one), malignant carcinoid of unknown origin (one), and carcinoma of the tongue (one), parotid (one), appendix (one), and anus (one). Only the patient with widely metastatic carcinoid had an objective response in a skin metastasis noted 6 weeks after initial treatment at a daily dose of 1000 mg/m^2. Disease progression at other sites was documented 6 weeks after a second course at the same dose despite continuing improvement in the responding skin lesion.

DISCUSSION

Because of unpredictable severe diarrhea occurring in three of six patients receiving PALA at a dose of 1250 mg/m^2/day × 5 consecutive days, we were reluctant to explore higher doses as initial treatment of advanced cancer. Two of the three patients with diarrhea had significant ascites. Although doses were calculated on estimated dry weight, it is possible that these were overestimates and resulted in greater toxicity. Alternatively, fluid retention itself may accentuate toxicity by delaying elimination of the drug.

For subsequent phase II evaluation of PALA, we recommend a starting dose of 1250 mg/m^2/day × 5 days with consideration being given to modest dose escalation (20%) in patients experiencing no toxic effects. It appears that courses of PALA may be repeated every 3–4 weeks except in the occasional patient with myelosuppression who has not fully recovered at the time of re-treatment.

We had no experience in treating patients with significant impairment of kidney function in this study. Since the major route of excretion of PALA in man is renal,[5] consideration should be given to reducing the dose of initial treatment given to patients with impaired renal function.

In a subsequent phase II study of PALA in patients with advanced large bowel carcinoma, five have received a dose of 1250 mg/m^2/day × 5 days without experiencing significant gastrointestinal or hematologic toxic effects. One of these patients had an erythematous pruritic rash involving the axillae, breast folds, and labia majora starting 2 weeks after treatment and lasting for 1 week. Three patients have been re-treated at the same dose 4 weeks after their initial treatment. Their courses are not evaluable as yet.

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Cyclocytidine in the Treatment of Refractory Acute Childhood Leukemia: A Southwest Oncology Group Phase I–II Study [1,2]

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SUMMARY

Thirty-seven children and adolescents with acute leukemia in relapse were treated with cyclocytidine in a cooperative group setting. Only one of the 27 evaluable patients achieved complete remission. A significant decrease (≥ 20%) in circulating and/or bone marrow leukemia cells occurred in an additional five patients. Drug toxicity was evaluated in 35 patients and included ten cases of jaw pain, two cases of hypotension, one case of fever, and one case of severe vomiting.

[Cancer Treat Rep 63:1913–1915, 1979]

Cytosine arabinoside (Ara-C) has been utilized extensively for the treatment of hematologic malignancy in patients in all age groups (1). It is desirable to maintain plasma levels for prolonged periods to maximize the antineoplastic potential of this agent since it is phase-specific, ie, it is cytotoxic only during the S phase of the cell division cycle (2,3). However, this requires continuous iv infusion since this drug is metabolized within minutes by pyrimidine nucleoside deaminases (2,3). Investigators in both Japan (4) and the US (5) have suggested that cyclocytidine may circumvent this problem since it is resistant to deamination. Pharmacodynamic studies have shown that plasma levels of Ara-C are maintained for ≥ 6 hours after cyclocytidine administration, as a result of the relatively slow hydrolysis of cyclocytidine to Ara-C (6).

Although cyclocytidine has shown promise in the treatment of acute nonlymphoblastic leukemia in clinical trials in Japan (7), clinical trials conducted in adults in this country have been disappointing (8). Since clinical response to cyclocytidine in acute childhood leukemia remained to be elucidated, we conducted a phase I–II clinical trial in this disease in a cooperative study group setting. We were interested in whether patients would be cross-resistant after the development of insensitivity to Ara-C and whether side effects such as postural hypotension would render impractical the outpatient use of this agent as a depot form of Ara-C in children and adolescents.

PATIENTS AND METHODS

Thirty-five children and adolescents (< 21 years of age) with acute leukemia in relapse, with > 25% blast cells in the bone marrow, were considered eligible for this clinical trial which was conducted from July 1975 through September 1977. Two patients were considered ineligible due to insufficient data. Thirty-one patients had the diagnosis of acute lymphoblastic leukemia and four had the diagnosis of acute myeloid leukemia. This was established by standard morphologic criteria (1). Twenty-nine of 35 eligible patients had been previously treated with Ara-C (table 1). All patients had a complete medical history, physical examination, and cbc and blood chemistries including liver and renal function tests before each course

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TABLE 1.—Previous Ara-C therapy in 35 eligible patients

	No. of patients
Type of previous Ara-C therapy	
None	5
Intrathecal only	2
Systemic	14
Systemic + intrathecal	13
Unknown	1
Total	35
Resistance to Ara-C	
Documented by records	19
Probable resistance to Ara-C	4
Sensitive to Ara-C	8
Ara-C resistance status unknown	4
Total	35

of treatment and, if possible, after the study was discontinued. Bone marrow aspirates and/or biopsies, performed at the beginning of the study and every 21 days thereafter, were utilized to document the remission status of the disease. Informed written consent was obtained from patients and/or their parents in accordance with the policies of the participating institutions.

Cyclocytidine was reconstituted with sterile water for injection, normal saline, or 5% dextrose solution at a concentration of 100 mg/ml and was considered stable for 12 hours after reconstitution. An initial 10-day course of therapy was given either sc or iv at a dose of 360 mg/m^2. The iv route was utilized exclusively in patients with severe thrombocytopenia (platelet count $< 25{,}000/mm^3$). Nineteen patients received the drug iv, 12 received the drug sc, and four received the drug by both of these routes. The iv dose was given by 1-hour infusion in three patients, by 2-hour infusion in one patient, and by "slow" iv push in two patients, and the timing of the iv infusion was not specified in the remaining 17 patients.

Blood pressure measurements were required in the upright and prone positions before administration of the drug, every 15 minutes during the first 2 hours, every 30 minutes during the next 4 hours, and then every hour thereafter until 16 hours after drug administration. Significant hypotension ($\geq$ 35% from baseline values) was a cause for withdrawal from the study.

Complete remission was defined as $\leq$ 5% blast cells in bone marrow aspirates of a minimum of 200 cell differential counts and normal myeloid and erythroid precursors. Partial remission was defined as 5.1%–25.0% blast cells in bone marrow aspirates or the disappearance of significant organ enlargement due to infiltration during therapy. Patients were removed from the study if they failed to achieve at least partial remission after two courses of treatment or if they demonstrated progressive disease after one course. Progressive disease was defined as a significant increase ($\geq$ 30%) in the percentage of leukemic blast cells in the bone marrow and/or peripheral blood. For patients who achieved remission, adequate granulocyte ($\geq 1000/mm^3$) and platelet ($\geq 100{,}000/mm^3$) counts were required before each course of treatment. In order for a patient's treatment to be considered evaluable, the effect of at least one full course of treatment must have been quantified in terms of either peripheral blood or bone marrow parameters. This implied a minimum survival of 10 days. A patient must have received a minimum of three doses of drug to be considered evaluable for toxicity.

RESULTS

Only one of the 25 evaluable patients with acute lymphoblastic leukemia achieved a transient complete remission of only 2 weeks and a partial remission of 15 weeks after being treated with cyclocytidine. Both evaluable patients with acute myeloid leukemia failed to respond, demonstrating continued presence of leukemic myeloblasts in the peripheral blood. One of these patients died 5 days after completing one and one half courses of treatment with severe thrombocytopenia and secondary hemorrhage. A significant decrease ($\geq$ 20%) in circulating and/or bone marrow leukemia cells was noted in an additional five patients with acute lymphoblastic leukemia. However, none of these latter patients attained partial remission and the duration of these responses was short (mean, 3.6 weeks; range, 3–6 weeks). It is noteworthy that no prior Ara-C had been administered systemically to the patient achieving remission. However, all five patients achieving improved hematologic status and 17 nonresponders had received previous systemic courses of Ara-C. Twenty-three of 35 patients were considered to be resistant to Ara-C (table 1).

Eight patients could not be evaluated for response for various reasons: early death before completion of one course of treatment (three patients), major protocol violations (two), insufficient data (one), intolerable toxicity (one), and refusal of further therapy (one). The major protocol violations involved the administration of additional chemotherapeutic agents immediately before or during the course of cyclocytidine.

The evaluable patients had received many previous induction chemotherapy courses (mean, 6.5; range, four to 13) before the start of the cyclocytidine study. Two evaluable patients received slightly higher dose schedules of 400 and 446 mg/m^2, respectively, but both of them failed to respond. All of the other patients received 360 mg/m^2/day, with no dose escalation in those receiving multiple courses of cyclocytidine. Twenty-one patients received only one course of treatment, two received two courses, and four received four to five courses.

Thirty-five patients were evaluated for toxicity. Se-

vere toxicity was observed in only one of these patients. This patient had to be removed from the study due to severe hypotension (> 35% reduction in systolic blood pressure) which developed within 1 hour of his sc injection and persisted at a level of approximately 70/30 mm Hg for 3–4 hours. Ten cases of mild to moderate jaw pain and single cases of moderate hypotension (≥ 15%, ≤ 35% decrease in systolic blood pressure), vomiting, and fever were noted in other patients evaluable for toxicity. Jaw pain began ≤ 2 hours after injection and persisted for ≤ 48 hours after cyclocytidine treatment was discontinued. No correlation between toxicity and abnormal liver or renal function tests was noted. It was not possible to assess hematologic toxicity, since the myelosuppression observed might have resulted from either cyclocytidine or leukemic infiltration. However, granulocyte and platelet counts either increased or remained normal in the six responding patients. Of the 35 patients treated, 28 received supportive care with parenteral antibiotics, 17 received platelet transfusions, and one received granulocyte transfusions.

DISCUSSION

The relatively low remission rate in our study is even less than that previously reported in patients with acute leukemia who received Ara-C as the sole induction agent (1). The primary advantage of clinical utilization of cyclocytidine compared to Ara-C is the convenience of administration, circumventing the need for continuous iv infusion. However, the frequency of various toxic effects and the need to monitor the patient carefully after each injection seemed to minimize this therapeutic advantage. The jaw pain and postural hypotension observed appear attributable to the beta-adrenergic action of this drug and can be antagonized by either propranolol or sympathectomy in animals (9,10). Nevertheless, it appears that alternative methods of prolonging the pharmacologic action of Ara-C may prove to be more practical clinically. These may well include the further development of agents (for example, rhodium[II] acetate) which inhibit degradation by cellular deaminases (11).

We conclude that cyclocytidine has limited value in refractory childhood leukemia at a dose level of 360 mg/m^2/day given in 10-day courses. Moreover, the potential toxicity markedly limits the clinical usefulness of this drug as a depot form of Ara-C in children and adolescents.

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The Delta and Epsilon Errors in the Assessment of Cancer Clinical Trials [1,2,3]

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SUMMARY

The error probabilities α and β are widely used to compute sample sizes and to analyze results of clinical trials. These errors are, however, not the only probabilities to consider when assessing results of clinical studies. The rate of false positive (δ) and false negative (ε) results allows one to determine if an experimental finding is likely to reflect the true situation in the population of interest. The δ error is generally high in randomized phase III and in early phase II clinical trials in cancer patients, whereas the ε error is relatively low in these settings. This is essentially due to the small probability of detecting a more effective treatment or a new chemotherapeutic agent active in cancer. The δ error could be considerably reduced by increasing the sample sizes and by restricting the allowance made for the α error, which should be set at a 1% level as a minimum requirement.

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Reproducibility of data is a prime requirement for arriving at convincing conclusions from adequately performed experiments. Cancer clinical trials have often yielded conflicting reports as illustrated by the wide range of response rates observed with 5-fluorouracil in colorectal cancer (1) or bleomycin in head and neck cancer (2). These inconsistencies have been related to a number of variables including primarily the selection of patients, the definition of end-point events, and the method of data reporting (3,4).

Assessment of clinical trials must also take into account two types of errors that are inherent in the statistical design of these trials. The type I error, also called the α error, is the error made by declaring a significant difference between two treatments when it does not exist. The type II error, or the β error, represents the failure of recognizing a significant difference between samples when it is truly present in an overall population. These errors can be misleading when assessing results of clinical trials because they suppose that the effectiveness of the new treatment relative to the control is known, whereas this is precisely what the trial attempts to elucidate.

The ratios of false positive trials to the total number of positive trials and of false negative trials to the total number of negative trials represent Bayesian probabilities which could be used to judge if significant or nonsignificant results achieved in a trial are likely to reflect the true situation in the population of interest (5). These probabilities are called here the δ and ε errors respectively. The use of posterior probabilities in clinical medicine has been very fruitful in the statistical evaluation

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of diagnostic tests (6–11). They may have a significant impact on the planning and analysis of cancer clinical trials.

PROPERTIES OF δ AND ε ERRORS

Suppose that 1000 trials with two equivalent treatments are performed with random samples from a given population (table 1). The decision rule for significance is set in such a way that 5% of the trials will show a significant difference ($\alpha = 5\%$). In this case, 50 trials will lead to a false positive conclusion. A type II error, with β equal to 50% for example, would lead to 500 studies with incorrect (false negative) conclusions in 1000 randomized trials with two treatments of different effectiveness.

Table 1 shows that two other errors may be easily computed by reading the data vertically. The error in positivity (δ error) is defined by the number of false positive trials over the total number of positive trials (50/500). The error in negativity (ε error) is found by calculating the ratio between the number of false negative trials and the total number of negative trials (500/1450). δ and ε are related to α and β in the following way (6):

$$\delta = \frac{(1 - \text{TER})\,\alpha}{(1 - \text{TER})\,\alpha + \text{TER}\,(1 - \beta)}$$

$$\varepsilon = \frac{(\text{TER})\,\beta}{(\text{TER})\,\beta + (1 - \text{TER})\,(1 - \alpha)}$$

where TER (true effectiveness ratio) is the ratio of true positive results to the total number of situations investigated (1000/2000 in table 1). Thus, both δ and ε increase with increasing α and/or β and decrease with decreasing

TABLE 1.—Theoretic outcome of a series of randomized trials

	Conclusion of the trial	
	Positive: significant difference in effectiveness	Negative: no significant difference in effectiveness
1000 trials with 2 treatments of equivalent effectiveness (Ho is true)	50 (false positive) ($\alpha = 0.50$)	950
1000 trials with treatments of different effectiveness (Ho is not true)	500	500 (false negative) ($\beta = 0.50$)
	550	1450

δ = 50/500 = 0.09. ε = 500/1450 = 0.34.

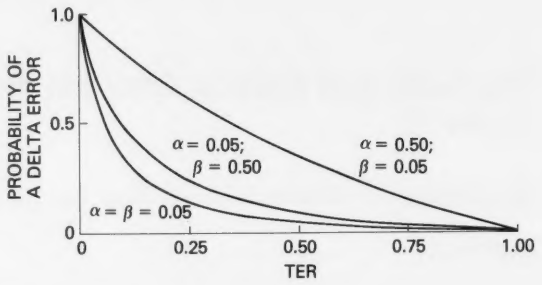

FIGURE 1.—Rate of false positive trials (δ error) as a function of the TER and the size of α and β.

α and/or β. In addition, slight variations in TER may result in enormous changes in δ and ε errors even when α and β errors are kept constant. A reduction in TER augments δ and lowers ε and vice versa (figs 1 and 2, table 2). When TER is < 0.5, the most significant effect is seen on the δ errors but this effect is somewhat tempered by a progressive diminution in the proportion of trials yielding positive results when TER is diminishing (fig 3).

δ AND ε ERRORS IN CANCER CLINICAL TRIALS

This section will discuss the effect of δ and ε errors in classic phase III randomized comparative trials and in early phase II nonrandomized trials.

Phase III Trials

In most phase III trials a new treatment is compared to another treatment or to no treatment after random allocation of the patients. Usually, the design of such trials is such that the level of the α error is preselected at 5% ($P < 0.05$) whereas much less uniformity is seen for the level of the β error. In randomized trials, the TER

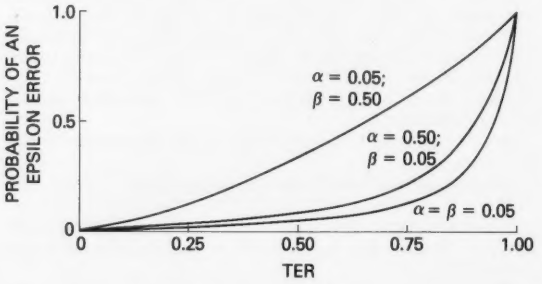

FIGURE 2.—Rate of false negative trials (ε error) as a function of the TER and the size of α and β.

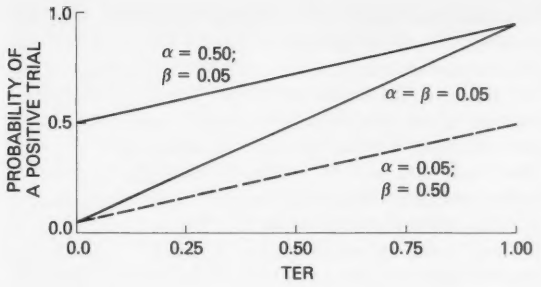

FIGURE 3.—Probability of achieving a positive result in a trial as a function of the TER and the size of α and β.

provides the a priori probability that the new treatment is better than the control. This figure can be estimated from past experience.

Few randomized trials have actually demonstrated a superior efficacy of a new treatment compared to the reference therapeutic approach (12). However, a majority of the reported randomized trials have used small sample sizes resulting in a lack of sufficient power (1 – β) to detect true differences (13–15). Moreover, it may be difficult to determine retrospectively from published results if a particular trial has been completed or is still ongoing and if the initial purpose was to demonstrate a difference in efficacy. Nevertheless, a critical analysis of published randomized trials in a variety of tumor types (12) indicates that the value of TER can reasonably be assumed to fall into a range of 1%–20%. Under these conditions, the ε error is acceptable in most cases but the δ error may reach extremely high levels (table 2). When a therapeutic difference is reported to be statistically significant there is at least a 17% chance that this difference does not exist with α at 5% and a power (1 – β) of 95%. This figure is further but moderately worsened when the power of the test declines as exemplified with β = 50% (table 2).

It can be easily calculated that, in the favorable case of TER = 20%, the α error must be, in fact, set at 1% at most, for a β = 5%, in order to keep the δ error below the 5% limit. This requirement would necessitate a doubling of the total patient accrual as compared to a design using α and β = 5%.

Phase II Trials

Several authors (16,17) have investigated the optimal sampling strategy to use in early phase II clinical trials for new anticancer drugs. The requirements are that a decision to continue using a new drug be reached with a minimum number of patients and with a low rate of false negativity and false positivity. It is generally agreed among clinical investigators that a new substance must produce a response in at least 20% of the diseased population to be accepted for further study. In this paper a drug will be called ineffective if it does not fulfill this definition. This is valid for tumor types refractory to chemotherapy and has also become relevant for more sensitive tumors since these are treated with investigational agents only after progression from prior combination chemotherapy is documented.

Phase II trials are designed in such a way that the probability of rejecting an effective drug (β) is kept below 5% (16) because it is felt that such a mistake is much more serious than accepting an ineffective therapy. Keeping the sample size small may result in a very high false positive rate. For instance, when investigating a drug in 14 consecutive patients and accepting this drug for further study, if one response is seen there is a 51% chance of accepting a drug with only a 5% true activity rate (18).

As seen earlier, the magnitude of the δ and ε errors depends upon the α and β errors and upon the TER which here is the a priori probability that the investigational agent is effective. Assuming that TER is 1/10, let us suppose, then, that two effective drugs and 18 ineffective drugs have been submitted to phase II trials in 14 consecutive patients with a particular tumor type (table 3). The absolute figures are not important in the table since only ratios are relevant to δ and ε errors. Computation of these errors indicates that 83% of the tests declared positive are in fact negative and that 1% of the negative tests are in fact positive (table 3).

Only a slight improvement is seen if one increases the sample size to 30, with the decision rule that a drug will be submitted to further testing if at least three responses are seen, the definition of effectiveness being the same as above. In this case, the β error (false negativity) remains approximately 5%, and the α error is reduced to 19%. By constructing a 2 × 2 table, it can be calculated

TABLE 2.—δ and ε errors at selected levels of TER and α and β errors

	α = β = 5%		α = 5%, β = 50%		α = 50%, β = 5%	
TER	δ (%)	ε (%)	δ (%)	ε (%)	δ (%)	ε (%)
0.20	17	1	29	12	68	2
0.10	32	0.60	47	6	83	1
0.01	84	0.05	91	0.53	98	0.10

TABLE 3.—Theoretic outcome of a series of 20 phase II trials with 14 consecutive patients with a particular tumor type

	Conclusion of the trial	
	Effective†	Ineffective
18 truly ineffective drugs	9.2 (α = 0.51)	8.8
2 truly effective drugs	1.9	0.1 (β = 0.05)
	11.1	8.9

δ = 9.2/11.1 = 0.83. ε = 0.1/8.9 = 0.01.

†An effective drug is defined as one which gives a response rate $\geq$ 0.2 in a given population.

that the δ error becomes 64% and the ε error becomes 0.60%.

A series of curves can be constructed for several TERs and sample sizes showing that the rate of the δ error is extremely high for TERs < 1/10.

DISCUSSION

The usual statistical rules used to analyze results of clinical trials in cancer are such that they favor a high rate of false positive results when significance is reached. The δ and ε errors as described here consider not only the α and β probabilities but also the probability that an active or a more active treatment becomes available, which is expressed by the TER. This ratio will obviously never be known with precision, but computations can be made over a range of its values giving an "operating characteristic curve" for a given trial (figs 1 and 2). By examining such a curve, it is then possible to qualify any positive trial by taking into account all the available past information. In some diseases, valid estimations of TER are not available because most of the trials have a limited power.

Cancer clinical trials are most likely to deal essentially with TER values of about 0.10. In this case, the error in negativity (ε) is generally small even if the power of the test is not > 50%. In contrast, the error in positivity (δ) may become extremely high unless huge numbers of patients are accrued. This situation reemphasizes the need for solid rationales before embarking on clinical trials. Clearly, the highest priority must be given to testing the major principles of therapy and the investigation of minor questions probably should merely be avoided. The puzzling magnitude of the δ error also calls for the greatest care when designing clinical trials. Adequate questions and proper design not only are more consistent with large accrual of patients, but they also are instrumental in increasing the TER.

Randomized trials have been widely advocated because, in principle, as compared to nonrandomized trials, this design circumvents problems associated with the variability in the selection of patients, response criteria, and method of data reporting. In addition, as pointed out by Byar et al (19), they also allow a stronger statistical analysis of the data. However, ethical considerations are not consistent with comparisons of treatments of apparently very different effectiveness, resulting in a necessarily low TER. Thus, only negative results will have a reliable validity provided that a sufficient power is used. In fact, it may be calculated that phase III trials should involve sample sizes of several hundreds of patients. Hence, negative findings in randomized trials with large sample sizes appear to be a most satisfactory demonstration that two therapeutic approaches are equivalent in terms of efficacy.

The large proportion of false positive results in randomized trials points to the inadequacy of selecting a level of 5% for the α error. Although a reliable figure cannot be accurately ascertained, it appears that a 1% level for α would represent a minimum requirement. However, it would not prevent confirmatory trials from being mandatory. This pertains even more strongly to cases where trials are terminated because a significant difference is observed before having accrued the total predetermined number of patients. Eventually, the use of unbalanced randomization would obviate ethical doubts in subsequent trials by giving the supposedly more active treatment to a larger number of patients.

Similar problems are encountered in the nonrandomized phase II trials with investigational anticancer agents. A TER value of 0.10 seems a reasonable approximation in this case, considering that true effectiveness pertains to usually refractory tumor types or to tumors resistant to conventional chemotherapy. Consequently, the ε error will remain very small indicating a high reliability, from a statistical standpoint, of negative trials, provided that the method and rationale for introducing new agents into clinical investigation remain unchanged.

It has been shown that the δ error may be extremely high in classic phase II studies, but this observation is of much less importance than it is in the case of phase III trials. First, the values tabulated in our examples relate to situations where only one response is seen in 14 consecutive patients or only three responses are seen in 30 consecutive patients. Obviously, the δ error can be noticeably reduced when more responses are achieved, ie, > 1/14 or > 3/30. In addition, clinical investigators are usually aware of the high rate of the α error in these trials and the procedure of using, for example, 14 patients in an initial screen is usually part of a two-stage design for phase II studies (16,18).

Our observations do not contribute to the controversies of randomized versus nonrandomized trials. However, they indicate that results of positive trials, ie, trials showing a difference between treatment options, cannot be

properly interpreted on the basis of their face value. So far, this has been barely recognized by the advocates of the randomized procedure.

The Bayesian approach described in this paper is not widely accepted in clinical research, due to the subjectivity in determining the a priori probability of success. Although other considerations than TER undoubtedly play a role in designing clinical trials and analyzing clinical data, this method gives one possible explanation for nonreproducible results and underlines the importance of conducting confirmatory trials before embarking on large-scale use of new treatment modalities.

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Role of Cholesterol in Enhancing the Antitumor Activity of Cytosine Arabinoside Entrapped in Liposomes [1,2]

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SUMMARY

Studies were carried out to determine whether there was any correlation between liposome composition, permeability of entrapped drug, and antitumor activity against mouse L1210 leukemia. These studies were done using reverse evaporation phase liposomes composed of the phospholipids phosphatidylglycerol (PG) and phosphatidylcholine (PC) in a constant molar ratio (1:4) and with varying amounts of cholesterol (Chol). The results indicated that with increasing amounts of Chol present in the liposomes (from a total absence of Chol to a molar ratio of 1:4:5 of PG:PC:Chol) the rate of leakage of cytosine arabinoside (ara-C) was markedly reduced. At 24 hours following in vitro incubation of liposomes containing entrapped ara-C in the presence of 80% fetal calf serum, the amount of ara-C remaining associated with the liposomes was 72% with liposomes composed of PG:PC:Chol (at ratios of 1:4:5) but was reduced to 12% with liposomes composed of only PG:PC (at ratios of 1:4). Furthermore, increased molar ratios of Chol:phospholipid correlated with an increase in the antitumor activity by ip injection against ip inoculated mouse L1210 leukemia. Significant numbers of mice surviving 30 days were observed only when Chol was present in PG:PC:Chol ratios of 1:4:3 and 1:4:5. These results suggest that reverse evaporation phase liposomes can be made which are relatively impermeable in serum by manipulation of the Chol:phospholipid ratio and thus can be relatively stable in biologic fluids. In addition, the results indicate that the antitumor activity of the entrapped drug is inversely related to its permeability through the liposome membrane.

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Liposomes (phospholipid vesicles) have been the subject of a number of investigations as a drug-delivery system in vitro and in vivo (1–3). While there have been many reports suggesting their potential as a drug carrier (4,5), there have been relatively few where significant in vivo effects have been demonstrated and confirmed.

Several investigators (6–10) have shown that entrapment of cytosine arabinoside (ara-C) in liposomes significantly potentiates this drug's activity against L1210 tumor in mice. Circumstantial evidence has suggested that the in vivo effects may be due to a "sustained release" or "depot" action of the liposomes (2,7,8,10), although the possibility of "local release" or specific delivery of drug *in liposomes* into the target cells is not excluded. Irrespective of the mechanism of cellular uptake, the rate of loss of ara-C from the liposomes must play an important role in the therapeutic effectiveness of the drug-liposome combinations.

In this report, we compare the antitumor activity against L1210 leukemia of ara-C entrapped in liposomes, prepared by the recently described method of reverse

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phase evaporation (11), composed of similar phospholipids but varying in ratio of cholesterol (Chol) to phospholipid. The size of the resultant liposomes was controlled by extrusion through membranes of defined pore diameter (12). The rates of leakage of ara-C from the same liposomes in vitro in the presence of serum were also determined and were compared with the antitumor effects. These studies were done to simulate some of the effects of the biologic environment on liposomes in vivo.

MATERIALS AND METHODS

Lipids and Other Materials

Chol was purchased from A. B. Fluka (Buch, Switzerland) and was recrystallized twice from methanol. Phosphatidylcholine (PC) was purified from egg yolk by a modification of previously described methods (13). Total phospholipid extract from fresh egg yolks was passed through an activated alumina column and eluted with chloroform:methanol (1:1) to separate PC from phosphatidylglycerol (PG). The partially purified PC was then applied to a silica gel column (Prep Pak 500, Waters Associates, Milford, MA) on a Waters Associates preparative LC-500 liquid chromatograph. The column was pressurized to 600 psi with nitrogen and equilibrated with chloroform:methanol:water (60:30:4) at a flow rate of 200 ml/minute until a stable baseline was obtained. In a typical preparation, 25 g of semipurified PC in chloroform:methanol:water (60:30:4) was applied to the column, impurities were removed in the first 600 ml of solvent, and the purified PC was eluted in the next 1200 ml. The elution of the PC from the silica gel column was monitored by the differential refractometer on the instrument and by thin-layer chromatography on silica gel 60 (No. 5762, E. Merck, Darmstadt, Germany) in chloroform:methanol:7 M NH_4OH (230:90:15). The spots were visualized by using molybdate and by charing with sulfuric acid (14). The yield of pure PC was typically 50%. PG was synthesized by transesterification of egg PC by cabbage phospholipase D as previously described (14). The crude mixture contained egg phosphatidic acid, PC, and impurities from the enzyme in addition to PG. The silica gel column of the LC-500 preparative was pressurized to 600 psi and equilibrated with chloroform:methanol:water (60:20:1) at a flow rate of 200 ml/minute until a stable baseline was obtained. The crude egg PG was injected onto the column in 50 ml of chloroform and eluted with the above solvent. The first 600 ml contained phosphatidic acid; the next 1000 ml contained PG with a water-soluble impurity which was removed by washing under nitrogen with a solution of 0.1 M ethylenediamine tetraacetic acid (pH 9.0) and 0.1 M NaCl. The wash solution contained chloroform:methanol:water in a ratio of 10:5:3. The organic phase was washed two additional times with 0.1 M NaCl. After the final wash an adequate amount of 100% ethanol was added (to help remove the water) and the purified PG was evaporated to dryness, redissolved in chloroform, filtered through Whatman No. 1 paper under nitrogen, and stored under nitrogen at –50°C. The phospholipids were > 99% pure by thin-layer chromatography, and were stored in chloroform in sealed ampules under nitrogen at –50°C until use. Ara-C was obtained from Sigma Chemical Corp, St. Louis, MO, and ^{3}H-ara-C was obtained from Amersham-Searle, Arlington Heights, IL. ^{14}C-Chol was obtained from New England Nuclear Corp, Boston, MA. Polycarbonate membranes (0.4-μ pore) were obtained from Nucleopore, Pleasanton, CA. Fetal calf serum was obtained from GIBCO, Grand Island, NY. Diethyl ether was redistilled from sodium bisulfite immediately before use to remove peroxides.

Preparation of Liposomes

Liposomes were prepared as previously described (11). The phospholipids with the appropriate ratio of Chol were mixed in chloroform, and the solvent was removed on a rotary evaporator depositing the lipid on the sides of a 100-ml round-bottom flask. The lipids were then redissolved in diethyl ether (3 ml for 60 μmols of total lipid) and then 1 ml of a 300 mM ara-C solution (pH 7.4) per 3 ml of diethyl ether was added. The flask was flushed with nitrogen and sonicated in an 80-watt bath-type sonicator (Lab Supply, Hicksville, NY) for 5 minutes at 5°C–10°C. The homogenous emulsion formed by the sonication was placed on a rotary evaporator and the organic solvent was removed under reduced pressure (water aspirator) at 20°C–30°C. Following removal of the diethyl ether, the opalescent suspensions of liposomes were extruded through a 0.4-μ polycarbonate membrane to form more uniformly sized preparations (12). Residual diethyl ether and non-encapsulated ara-C were removed by dialysis against five changes of 100 volumes of phosphate-buffered saline (PBS) (6) without calcium and magnesium (–Ca Mg) overnight. A typical preparation resulted in the encapsulation of 30%–35% of the initial ara-C present. Control liposomes were made under the same conditions except that ara-C was substituted with dextrose at the same concentrations and molar ratios (1:4:5, PG:PC:Chol) of lipids.

Permeability Determinations

Aliquots of freshly prepared liposomes were added to 0.7-cm diameter dialysis bags containing PBS (–Ca Mg) and/or heat-activated (56°C, 1 hour) fetal calf serum, which was freshly dialyzed against PBS (–Ca Mg), to give a final concentration of 10 μmols of lipid/1-ml volume. The bags were dialyzed against PBS (–Ca Mg) at 37°C with

a change of dialysate at 1, 2, 4, 8, and 24 hours. The amount of radioactivity was determined in each dialysate and also in the liposome suspension inside the bag at the termination of the experiment. The results were calculated as the percent of ara-C remaining in the dialysis bags at each time point. In control experiments, it was shown (*a*) that non-entrapped ara-C, in the presence or absence of serum, dialyzed completely from the bags in 1 hour, and (*b*) that treatment of the liposome suspension with triton x-100 (0.1% vol/vol) also resulted in essentially complete loss of ara-C from dialysis bags within 1 hour in the presence or absence of serum. These control experiments indicate that retention of ara-C in dialysis bags occurs only when ara-C is not in a freely diffusible state, ie, entrapped in the liposomes. Fresh mouse, rat, or human serum (undialysed) gave results similar to heat-inactivated (dialyzed) calf serum.

Antitumor Activity Against Mice Bearing L1210 Cells

Female DBA/2 Jackson mice, 6–8 weeks old and weighing 18–20 g, were injected ip with 10^6 L1210 cells on Day 0. Twenty-four hours later, mice were randomized and injected ip with PBS, liposomes containing ara-C, liposomes containing free ara-C, or control liposomes mixed with free ara-C. Only dead mice were used to calculate the mean survival time and standard deviation. Experiments were terminated at 30 days.

Identification of Ara-C

Identification and determination of the concentration of the free and entrapped ara-C were carried out in selected experiments using a high-pressure liquid chromatographic system (15).

RESULTS

Effects of Cholesterol on the Permeability of Liposomes Containing Ara-C

Figure 1 shows the rate of leakage of ara-C from liposomes of different Chol:phospholipid ratios but a constant molar ratio of PG:PC (1:4) in the presence and absence of serum at 37°C. The data indicate the following: (*a*) During the first 4 hours of in vitro incubation of liposomes containing ara-C in the absence of serum, the rate of leakage was not affected by the presence of Chol. For example, 85% of ara-C remained associated with the liposomes without Chol (PG:PC, 1:4) and 88% remained associated with the liposomes with high Chol levels (PG:PC:Chol, 1:4:5). (*b*) At 24 hours, however, while 83% of ara-C remained associated with liposomes with high Chol levels (1:4:5), only 30% remained associated with liposomes without Chol. This stabilizing effect of Chol can be observed even at the lowest ratio of Chol:phospholipid examined and becomes more pronounced in the presence of serum (fig 1). (*c*) The times for 50% leakage of ara-C from different liposomes in the presence of 80% serum were approximately 1 hour for PG:PC (1:4), 4 hours for PG:PC:Chol (1:4:1.5), 20 hours for PG:PC:Chol (1:4:3), and > 24 hours for PG:PC:Chol (1:4:5). Hence, with an increasing ratio of Chol:phospholipid in the bilayer, the rate of leakage of ara-C from the liposomes was markedly reduced.

Antitumor Activity of Ara-C Entrapped in Liposomes

Table 1 summarizes the antitumor activity of ara-C entrapped in liposomes against mice bearing L1210 tumor cells. The data indicate that all ara-C-containing liposomes exhibited a dose-dependent antitumor activity. Moreover, the increase in antitumor activity was correlated with increasing amounts of Chol in the liposomes. The maximum antitumor activity for PG:PC liposomes (at 100 mg/kg) was less than that obtained with liposomes containing molar ratios of 1:4:1.5 of PG:PC:Chol (at 25 mg/kg) or with liposomes containing 1:4:3 or 1:4:5 molar ratios (at 10 mg/kg). Comparing the effects at 10 mg/kg, it can be seen that liposomes containing PG:PC:Chol ratios of 1:4:3 and 1:4:5 were considerably more effective than liposomes with ratios of 1:4:0 or 1:4:1.5. The maximum effects, considering long-term survivors, were found at a dose of 50 mg/kg with 1:4:5 liposomes. At 100 mg/kg, with 1:4:3 and 1:4:5 liposomes, some deaths not due to L1210 leukemia were observed at 7–10 days, indicating toxicity due to the treatment. These animals are included in the tabulated results. The data in table 1 also show that mixing free ara-C and control liposomes (1:4:5) did not produce significant increases in the lifespan of mice bearing L1210 cells.

DISCUSSION

It has been known for some time that including Chol in the bilayer reduces the permeability of liposomes to small molecules (16,17) and that this protective effect is particularly high in the presence of proteins which tend to increase the permeability of the liposomes (18). It has also been shown that the presence of serum induces a very rapid leakage of entrapped ions out of liposomes (19), and non-Chol-containing liposomes may break down rapidly by interaction with serum components (20). The data presented in this report show that increasing the Chol:phospholipid ratio in ara-C-containing reverse evaporation phase liposomes improved antitumor activity while simultaneously reducing the efflux of the entrapped ara-C from the liposomes.

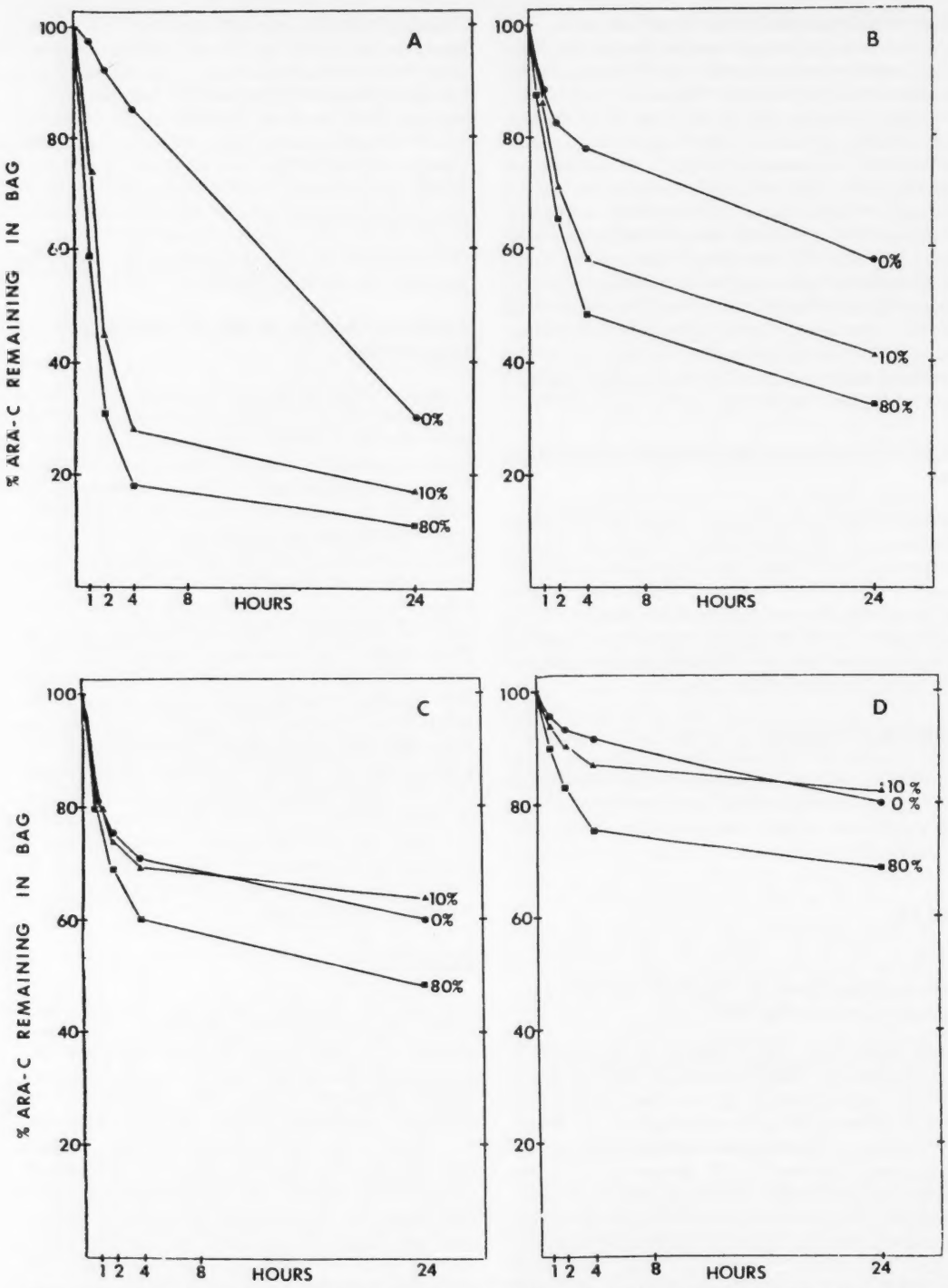

FIGURE 1.—Permeability of ara-C entrapped in reverse evaporation phase liposomes containing different amounts of Chol. Lipid compositions were as follows: A = PG:PC, 1:4. B = PG:PC:Chol, 1:4:1.5. C = PG:PC:Chol, 1:4:3. D = PG:PC:Chol, 1:4:5. % notations indicate the final serum content in the bag. Ordinate: % ara-C remaining in bag. Abscissa: time of incubation at 37°C. These data are from a single representative experiment. Other experiments gave similar results, with half-life of drug release that agreed to within 25%.

TABLE 1.—Effect of ara-C-containing liposomes composed of different Chol:phospholipid ratios on mean survival time of mice bearing L1210 leukemia

Treatment	Molar ratios of PG:PC:Chol			
	1:4:0	1:4:1.5	1:4:3	1:4:5
Ara-C liposomes				
10 mg/kg	11.9* ± 0.2 (0/16)	12.0 ± 2.0 (0/16)	18.3 ± 3.7 (0/16)	18.1 ± 2.6 (5/34)
25 mg/kg	13.7 ± 1.0 (0/16)	16.3 ± 4.7 (1/16)	21.0 ± 3.9 (5/24)	18.9 ± 1.1 (5/34)
50 mg/kg	12.4 ± 5.7 (0/36)	16.8 ± 4.8 (2/16)	17.9 ± 6.6 (5/16)	17.9 ± 4.9 (10/35)
100 mg/kg	14.6 ± 5.5 (1/26)	18.9 ± 6.5 (1/16)	15.5 ± 4.8 (4/24)	14.2 ± 2.6 (7/36)
Control (buffer only)	7.5 ± 0.1 (0/62)	—	—	—
Free ara-C (100 mg/kg)	8.4 ± 0.8 (0/16)	—	—	—
Free ara-C (100 mg/kg) + control liposomes (1:4:5)	ND†	ND	ND	8.4 ± 1.4 (0/16)

*Values = mean survival time (not including long-term survivors) ± SD. Nos. in parentheses = No. of mice surviving > 30 days/total No. of mice treated in 2–5 separate experiments.
†ND = not done.

Although the increased antitumor effectiveness of liposome-encapsulated ara-C has not been explained, it seems possible that ara-C entrapped in liposomes leaks out at a rate sufficient to result in a prolonged cytotoxic concentration of the active metabolite of ara-C (ara-CTP) (21) in target tumor cells. Since ara-C is active primarily against cells synthesizing DNA, the increased antitumor effects of liposomes containing a high Chol:phospholipid ratio may result from the slower release of ara-C, so that virtually all cells in the tumor population receive a cytotoxic dose of ara-C as they reach DNA synthesis. The rapid clearance by excretion and metabolism of free ara-C precludes any significant effects with a single non-encapsulated dose (21). It is unlikely that the higher concentration of Chol in the bilayer is affecting the L1210 cells by altering Chol content in their plasma membranes (22), since control liposomes composed of 50 mol% Chol in the presence or absence of 100 mg of non-encapsulated ara-C had no significant effect on the mean survival time of the animals (table 1). Furthermore, our own observations (unpublished data) indicate that Chol content does not significantly alter the total uptake of similarly sized negatively charged liposomes by L1210 cells in vitro or the initial tissue distribution of entrapped ara-C (and metabolites) in mice. Thus, it seems unlikely that the results could be due to factors other than the differential leakage properties of the liposomes, although it is possible that there are some differences in the pharmacokinetic behavior of the different liposomes.

The results are consistent with the supposition that the anti-L1210 effects in the present studies result primarily from a sustained release of ara-C from liposomes into the peritoneal cavities where the bulk of the tumor is present. However, liposomes and their contents do escape from the peritoneal cavities (2) and some systemic effects could be expected. Although in the present report we have used ip inoculated tumors and ip treatment regimens, we have shown that liposome-entrapped ara-C is effective after iv injection against iv inoculated tumor (8), and it is probable that similar effects of variation in Chol content could be observed in that system.

This work has some implications for the design of future studies of liposomes as drug carriers. It demonstrates the utility of having a well-characterized delivery system and the necessity of determining that liposome-encapsulated drugs remain encapsulated when in contact with biologic fluids. The results reported here show that liposomes can be made which are relatively impermeable in serum and thus can have a potentially long life in biologic fluids. Moreover, by manipulation of the Chol:phospholipid ratio as well as the specific phospholipid content, the leakage rate can be controlled in order to optimize the system for specific drugs in specific therapeutic applications.

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Phase I Trial of Bruceantin [1,2]

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SUMMARY

Bruceantin was administered to 33 patients with advanced solid tumors or hematologic neoplasms. The schedule consisted of four weekly iv injections at one dose level followed by a 2-week "no-treatment" period; this program was repeated every 6 weeks. A total of 156 weekly injections were given over a dose range of 0.8–8.5 mg/m^2. Systolic and diastolic hypotension was dose-related and dose-limiting at doses of $\geq$ 6.0 mg/m^2. Transient febrile reactions were dose-related. Gastrointestinal toxicity was commonly observed but was not dose-limiting. There was no hematologic or renal toxicity; hepatic toxicity was subclinical and reversible. Of 18 patients evaluable for antitumor response one patient with adenocarcinoma of the cervix experienced a $<$ 50% disease regression lasting 10 months and one patient with pleural mesothelioma experienced disease stabilization lasting 4 months. A phase II study of bruceantin is recommended at a starting dose of 5.5 mg/m^2 weekly for 4 weeks as a 4-hour iv infusion repeated at 6-week intervals.

[Cancer Treat Rep 63:1929–1932, 1979]

Bruceantin is a natural product obtained from alcohol extracts of the Ethiopian simaroubaceous tree *Brucea antidysenterica*. Kupchan and co-workers (1,2) initially isolated this simaroubolide in efforts to obtain antitumor compounds from plant sources. In animal systems, bruceantin increased the mean survival time of mice bearing P388 murine leukemia, B16 melanoma, colon tumor 38, and L1210 leukemia. [5,6]

Bruceantin irreversibly inhibits protein synthesis when tested in HeLa cells, rabbit reticulocytes, and reticulocyte lysates (3). Polyribosome function and peptide chain elongation are both inhibited in vitro when exposed to bruceantin. The dissociation of polyribosomes to monoribosomes has been observed after bruceantin administration. In HeLa cell systems, secondary effects on inhibition of DNA synthesis have also been noted. In preclinical large-animal toxicology studies, bruceantin resulted in acute and chronic gastrointestinal and hepatic toxicity. Specifically, dose-limiting bone marrow suppression, hypotension, and significant cardiovascular complications were not encountered. [7]

In October 1977, a phase I clinical study of bruceantin in both adult and pediatric tumor patients was initiated. The specific objectives were (*a*) to establish the qualitative and quantitative toxic effects caused by bruceantin and (*b*) to establish a dose level that was maximally tolerated with both predictable and reversible toxicity.

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[2]Supported in part by contract N01-CM-53839 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Division of Medical Oncology, Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA.

[4]We are grateful to Martha J. Sack for help in preparation of the manuscript.

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[6]Minutes of the meeting of the Phase I Working Group, Oct 7, 1976. Prepared by the Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, pp 67–80.

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MATERIALS AND METHODS

Patient selection.—All patients had histologic confirmation of a malignant disease which had become refractory to other therapies and for which there was no remaining therapy of proven clinical benefit. All patients were ambulatory, had an estimated survival of > 2 months, and had normal hematologic, renal, and hepatic functions. Objective measurable disease was not required. Written informed consent was obtained from all patients prior to treatment.

Treatment plan.—Bruceantin is formulated in 20-ml vials containing 3 mg/vial. The drug is reconstituted with 1.5 ml of absolute ethanol and is then further diluted with 13.5 ml of sodium chloride injection, 0.9% (USP), resulting in a solution of 0.2 mg/ml of bruceantin in 10% vol/vol of alcohol/saline. The proper dose was added to 250 ml of sodium chloride, 0.9% (USP), and was administered initially as a 30-minute rapid infusion through the side-arm of a running iv line. Because hypotension which was potentially both dose-related and dose-rate-related was observed at a later point in the study (vide infra), the drug was subsequently administered over a 4-hour time interval at doses of ≥ 6.0 mg/m^2. Bruceantin was given as a weekly injection for 4 consecutive weeks followed by a 2-week "no-treatment" period. Doses were escalated after the 6-week cycle. A single dose of bruceantin was defined as a single iv injection. A course of treatment was defined as one 6-week cycle of four weekly doses followed by a 2-week rest period. The starting dose of 0.8 mg/m^2/week for 4 weeks was extrapolated as a fraction of the lowest toxic dose of the most sensitive animal species.[8] After a minimum of three patients were evaluable, doses (in mg/m^2/week $\times$ 4 weeks) were escalated according to the following scheme: 0.8, 1.6, 2.8, 3.6, 4.4, 5.0, 5.5, 6.0, 6.5, 8.0, and 8.5. We projected six to eight escalations to reach the maximally tolerated dose (4). New patients were entered at the highest previously evaluated dose. Since no cumulative toxicity was observed in the preclinical toxicology studies,[8] doses were escalated within patients.

Study parameters.—Prior to treatment, patients underwent routine history and physical examinations with biochemical and radiographic documentation of extent of disease. The initial laboratory studies included cbc, BUN, creatinine, glucose, amylase, prothrombin time, transaminases, lactate dehydrogenase, alkaline phosphatase, calcium, phosphorus, total protein, albumin, bilirubin, urinalysis, and chest roentgenogram. Hepatic, hematologic, renal, and electrolyte studies were obtained prior to each treatment.

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Study termination.—Individual patients were removed from study when the maximally tolerated toxicity had been achieved and/or objective tumor progression had occurred. The phase I study was terminated when the maximally tolerated dose was established.

RESULTS

Thirty-three patients were entered in this phase I study, all of whom were evaluable for toxicity. Patient characteristics are shown in table 1. There were nearly twice as many females as males; the median age was 54 years (range, 5–70). Four patients received bruceantin as initial chemotherapy, including two patients with renal adenocarcinoma and one each with epidermoid carcinoma of the lung and pancreatic adenocarcinoma; the majority of patients had been heavily pretreated with chemotherapy, radiation therapy, or both. Thirty patients had metastatic solid tumors; three patients with hematologic neoplasms were treated, including two pediatric patients (5 and 6 years old) with acute lymphocytic leukemia and one adult patient with multiple myeloma. The categories represented by "other" in table 1 included one patient each with melanoma, adenocarcinoma of the cervix, pleural mesothelioma, medullary carcinoma of the thyroid, and adenocarcinoma of the bile duct.

Toxicity

There were several clearly definable and predictable toxic effects which were associated with bruceantin administration (table 2).

Hypotension.—Systolic and diastolic hypotension was both dose-related and dose-limiting at higher dose levels.

TABLE 1.—Phase I bruceantin study: patient characteristics

Characteristics	
Total No. of patients	33
No. of males/females	12/21
Age (yrs)	
Median	54
Range	5–70
No. of patients with—	
Prior treatment	
Chemotherapy	24
Radiation therapy	22
No prior treatment	4
Tumor types	
Lung	6
Genitourinary	6
Colon/pancreas	5
Breast	4
Head and neck	4
Hematologic	3
Other	5

TABLE 2.—Bruceantin toxicity

No. of patients	Dose (mg/m²/wk)	No. of doses*	% of doses causing— Hypotension†	Febrile reactions	Anorexia, nausea, and vomiting
4	0.8	15	0	7	0
5	1.6	18	0	17	17
3	2.8	10	0	30	20
9	3.6	27	0	22	37
6	4.4	19	0	21	32
8	5.0	21	0	76	57
3	5.5	12	0	67	33
3	6.0	9	11	89	44
6	6.5	15	40	80	67
1	8.0	3	0‡	100	67
2	8.5	7	0§	86	29

* Dose defined as 1 weekly injection.
† Defined as a drop in systolic pressure of ≥ 30 mm Hg and diastolic pressure of ≥ 30 mm Hg.
‡ 1 dose was associated with a mild decrease in blood pressure.
§ 3 doses were associated with a mild decrease in blood pressure.

Three patients received a total of nine doses of bruceantin at the 6.0-mg/m² dose level, administered over 30 minutes. One 54-year-old patient developed symptomatic hypotension during the first dose of bruceantin and was then removed from the study. The other two patients received four doses each and experienced no hypotension. Both of these patients (Nos. 1 and 2) had been given lower doses (2.8 and 3.6 mg/m², respectively) and had their doses escalated up to the 6.0-mg/m² level. Four of six patients (age range, 42–63 years) received bruceantin administered over 4 hours, at an initial dose of 6.5 mg/m². All four of these patients developed symptomatic hypotension in each of the six doses administered. Two patients were removed from the study after receiving the first dose of bruceantin at 6.5 mg/m² and two were removed from the study after receiving the second dose. The mean blood pressure in these four patients before bruceantin was 125/77 mm Hg; shortly after bruceantin administration, the mean blood pressure was 75/30 mm Hg. Blood pressure values reverted to normal approximately 12–14 hours after drug administration. Saline administration was given as specific treatment since all patients responded to this therapeutic maneuver. Vasopressors were not used. Self-limited atrial premature contractions developed in two patients during these hypotensive episodes at 6.5 mg/m². One patient (No. 1) received three doses of bruceantin at 8.0 mg/m², which was associated with only a mild decrease in blood pressure (≤ 30 mm Hg drop in systolic and diastolic) in one dose. Two patients (Nos. 1 and 2) received seven doses at 8.5 mg/m². Three of these seven doses were associated with a minor decrease in blood pressure. Both of the patients treated at the 8.0- and 8.5-mg/m² dose levels had had escalations up to this dose from lower levels and had not experienced any hypotension at a dose level of < 8.0 mg/m². Hypotension was not observed in any patient receiving a dose of < 6.0 mg/m².

Febrile reactions.—Overall, febrile reactions were noted in 70 of 156 doses (45%). Fever was observed at all dose levels and was dose-related (7% of 15 doses at 0.8 mg/m² versus 86% of seven doses at 8.5 mg/m²). The temperature elevation characteristically began 6–10 hours after drug administration, sometimes heralded by a chill, and lasted 6–7 hours thereafter; it occurred with similar frequency with both the 30- and 4-hour drug infusions. The temperatures were usually moderate (101°F–102°F) and occurred during the first exposure to the drug. Treatment with diphenhydramine ameliorated the chill symptoms. Fevers tended to be low-grade (99.5°F–100.5°F) following diphenhydramine administration. The prophylactic use of diphenhydramine was then employed routinely (50 mg orally 30 minutes prior to drug administration and every 6–8 hours for three to four doses thereafter) in patients receiving doses of ≥ 2.8 mg/m²/week. This procedure decreased the incidence of chills. In addition, the maximal temperature elevations were diminished with pretreatment diphenhydramine. The development of fever was a consistent feature in those patients experiencing hypotension (vide supra).

Other toxicity.—Gastrointestinal toxicity was observed in 55 of 156 doses (35%); although there was no clear dose relationship, the incidence was infrequent at doses of < 2.8 mg/m². Anorexia and nausea were the most common complaints and lasted 24–36 hours after drug administration. Vomiting, lasting 3 days, occurred in one patient treated at a dose of 2.8 mg/m² and necessitated drug discontinuation. Hematologic toxicity (rbc, wbc, and platelet toxicity; clotting) did not occur in any patient receiving bruceantin in the schedule employed. Extravasation of drug in one patient did not result in phlebitis

or local skin problems. Alopecia, which was mild and reversible, occurred in two patients receiving more than four doses of the drug at 5.5 mg/m^2. One patient with insulin-requiring diabetes mellitus required a doubling of the insulin dose after receiving bruceantin. Four patients (at various dose levels) developed transient leukocytosis following bruceantin administration which may have been related to temperature elevation. Renal toxicity was not observed. One patient experienced an acute febrile reaction (T = 103°F), which was accompanied by confusion, irritability, and a staggering gait 2 hours after receiving a dose of 5.0 mg/m^2. Neurologic examination was otherwise unremarkable. This symptom complex gradually resolved over a 2-week period. Subclinical and reversible elevations (less than two times normal) of bilirubin, transaminase, and alkaline phosphatase were noted and were not dose-related.

Antitumor Activity

Fifteen of the 33 patients evaluable for toxicity could not be analyzed for antitumor activity. Six patients were removed from study because of toxicity after receiving bruceantin and nine patients demonstrated progressive disease before receiving four weekly doses of drug. The 18 patients evaluable for response had the following diagnoses: lung carcinoma (three patients), colon carcinoma (three), breast carcinoma (three), genitourinary carcinoma (four), head and neck cancer (one), adenocarcinoma of the cervix (one), multiple myeloma (one), melanoma (one), and pleural mesothelioma (one). None of these patients achieved an objective 50% tumor regression. The one patient with adenocarcinoma of the cervix demonstrated a decrease in carcinoembryonic antigen from 57 to 7 ng/ml during therapy with a $< 50\%$ regression in her pulmonary nodules lasting for 10 months. The patient with pleural mesothelioma demonstrated stabilization of disease lasting > 4 months. These latter two patients had been previously treated with radiation therapy and chemotherapy prior to receiving bruceantin.

DISCUSSION

The toxicity of bruceantin has been described in 33 patients who received a total of 156 courses of the drug. Dose-limiting hypotension was noted in one patient initially treated at a dose of 6.0 mg/m^2 and in four patients initially treated at a dose of 6.5 mg/m^2. Patients who did not experience hypotension had been previously treated at lower doses and had dose escalations to and through the 6.0-mg/m^2 level. The etiology of the hypotension has not been elucidated. In preclinical studies with anesthetized dogs, bruceantin induced a decrease in cardiac output and an increase in peripheral vascular resistance, without changing arterial pressure (*see* footnote 7). The decrease in cardiac output in this animal study was secondary to a reduction in stroke volume which may have been mediated by a reduction in contractility of the myocardium. In the phase I study, hypotension was severe in five patients and necessitated drug discontinuation. There were no orthostatic changes in blood pressure. Saline administration was used to routinely treat these hypotensive episodes once they occurred; the prophylactic use of iv saline was not implemented in this study.

Gastrointestinal toxicity was generally self-limited and not strictly dose-related. Febrile reactions were dose-related and were ameliorated with diphenhydramine administration. There was no myelosuppression in patients receiving this weekly schedule.

There was no evidence of cumulative toxicity in the present study. One patient (No. 1) received a total dose of 120 mg/m^2 of bruceantin over a 12-month interval and demonstrated no evidence of cumulative toxicity. The patient had adenocarcinoma of the cervix and was removed from the study when a carcinoembryonic antigen titer, which had initially dropped, started to rise again.

The therapeutic activity of bruceantin cannot be assessed from this study because of the small numbers of patients. Based upon the defined toxicity, which was both reversible and predictable, the unique mechanism of action in animal and in vitro systems, and the lack of hematologic toxicity, a phase II study could be safely initiated at 5.5 mg/m^2/week for four doses every 6 weeks as a 4-hour iv infusion. Doses should be escalated to 6.0 and 6.5 mg/m^2 in those patients who tolerate the lower doses, with careful monitoring of arterial blood pressure.

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Phase I–II Trial of Methyl-GAG: A Southwest Oncology Group Pilot Study [1,2]

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SUMMARY

One hundred and nine patients with advanced malignancy, refractory to conventional therapy, were treated with a weekly schedule of methyl-GAG. Ninety-one patients (83%) were less than fully ambulatory. Sixty-five patients were fully evaluable for response. There were two complete and nine partial responses. An additional nine patients had objective tumor regressions and were classified as improved. The median duration of response was 5 months. Toxic effects were primarily mucositis (17 patients) and nausea and vomiting (14 patients). Sixty-seven patients had no manifestations of drug toxicity. Responses observed in a wide variety of solid tumors warrant further clinical trials.

[Cancer Treat Rep 63:1933–1937, 1979]

Shortly after Freedlander and French (1) described the growth inhibitory effects of methyl-GAG in mouse L1210 leukemia, it went through a phase of intensive preclinical and clinical investigation. Although the drug was found to have major activity in acute myelocytic leukemia on a daily dose schedule, its toxicity was protean: mucositis, vasculitis, and profound anorexia with associated weight loss; these effects were dose-limiting and often life-threatening (2–4). Various attempts made to improve the therapeutic index of methyl-GAG were not encouraging since most patients developed mucositis which appeared to be specific for methyl-GAG (5–7). Only the intermittent schedule as evaluated by the Eastern Cooperative Oncology Group and the Eastern Drug Evaluation Program appeared promising (8).[7] Responses were observed in carcinoma of the breast, esophagus, colon, rectum, oral cavity, and kidney and malignant lymphoma. Activity in esophageal cancer was subsequently reported by Falkson (9) using a daily dose schedule, but again, severe toxicity was observed. In the Eastern Cooperative Oncology Group study, however, toxicity was quite tolerable at dose levels of 6–8 mg/kg/week given im. Toxic effects consisted of anorexia, weight loss, and mild myelosuppression. Based on the activity and acceptable toxicity observed by the Eastern Cooperative Oncology Group and the Eastern Drug Evaluation Program, further evaluation of methyl-GAG on a weekly schedule was undertaken.

PATIENTS AND METHODS

Patients were eligible for entry in the study if they had a pathologically documented diagnosis of malignant disease, were not eligible for a study of higher priority within the Southwest Oncology Group, and had measurable disease. Minimum pretreatment evaluation included chest roentgenogram, liver and renal function tests, cbc with differential and platelet counts, and serum glucose. Pretreatment performance status was recorded

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[7]Livingston RB, Carter SK, Cooney DA, et al. Clinical brochure: methyl-GAG (NSC-32946). Prepared by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, Aug 1968, 38 pp.

for each patient in accordance with Southwest Oncology Group criteria.

Methyl-GAG was given as an iv infusion in 100 ml of 5% dextrose in water over ≥ 30 minutes. The initial dose was 250 mg/m²/week. This starting dose was subsequently increased to 500 mg/m² after the first 12 patients demonstrated no toxic effects and no response at 250 mg/m². In the absence of toxicity, the dose of methyl-GAG was increased by 100 mg/m²/week. The dose was reduced by 100 mg/m² if patients experienced moderate or severe reversible toxic effects. No change was made for mild toxicity.

The definitions of response were: *complete response*—the disappearance of all clinical evidence of disease; and *partial response*—reduction by at least 50% in the sum of the product of two perpendicular diameters of all measurable disease lasting for at least 4 weeks. Patients were considered to have improvement if measurable tumor regression occurred but did not meet the criteria for partial response.

RESULTS

Response.—One hundred and nine patients received at least one dose of methyl-GAG (table 1). Ninety-one of the patients (83%) began treatment while they were less than fully ambulatory. Thirty-six patients received only one or two doses of methyl-GAG: eight received 250 mg/m², three refused further therapy, seven were lost to followup, and 18 had early death (one drug-related and 17 caused by tumor). Two of the latter patients were exhibiting response to methyl-GAG when they died (one with renal carcinoma and one with hepatoma). Four patients received more than two doses of methyl-GAG at 250 mg/m². None of these patients had dose escalation or evidence of biologic effect. Four patients had protocol violations: two received simultaneous therapy with other agents and two had no measurable disease; one of these, a patient with colon cancer, had a > 50% decrease in carcinoembryonic antigen. Sixty-five patients who received more than two doses of methyl-GAG at 500 mg/m² were considered fully evaluable for response. Forty-four patients were not evaluable for response primarily because of extensive prior treatment and an extremely poor performance status which prohibited them from receiving more than two courses of drug. Among the evaluable patients, two had complete responses, nine had partial responses, and nine had improvement. Forty-five patients had no response to methyl-GAG. The median duration of response (complete plus partial) was 5 months. Four patients continue to respond: two with pancreatic carcinoma (7 and 8 months), one with non-oat cell lung cancer (7 months), and one with colon cancer (7 months). Of the patients responding to methyl-GAG, eight had dose escalations and 12 did not. The time to response was variable, with five patients responding after two courses, five responding after three courses, and ten responding after four or more courses. Response by diagnosis is summarized in table 2.

TABLE 1.—Characteristics of 109 patients receiving methyl-GAG

Characteristic	No. of patients
Methyl-GAG	
1 dose	109
1 or 2 doses	36
Dose not exceeding 250 mg/m²	8
Refused further therapy	3
Lost to followup	7
Early death	18
> 2 doses at 250 mg/m²	4
Protocol violation	4
> 2 doses at ≥ 500 mg/m² (Evaluable for response)	65

Toxicity.—Of the 109 patients treated, 12 received only 250 mg/m² of methyl-GAG (a dose which produced no apparent biologic effect) and seven were lost to followup. Information regarding toxicity is reported for 90 patients who received 500 mg/m² of methyl-GAG. Sixty-seven of these 90 patients (74%) had no toxic effects. The major toxic effect encountered was mucositis, which was seen in 17 patients (19%). One drug-related death secondary to mucositis was seen in a patient with hepatoma who received one dose of methyl-GAG at 600 mg/m². This patient had normal pretreatment hepatic and renal function, without ascites or effusion. The other prominent side effect was nausea and vomiting occurring in 14 patients (16%). Myelosuppression was seen in only seven patients (8%) and anemia was seen in only three (3%). The types and degrees of toxicity are summarized in table 3. Severe muscle pain and weakness of the lower extremities were seen in three patients; creatinine phosphokinase and aldolase levels were normal in all three. Muscle biopsy was not obtained. In each patient these effects were reversible with drug withdrawal. All three patients had received previous vinca alkaloids. One of these patients with acute leukemia developed this syndrome after three weekly doses of 1 g/m². Overall, toxicity appeared to be cumulative, since patients who received increasing numbers of courses experienced more toxic effects (table 4).

DISCUSSION

Previous trials of methyl-GAG given on a daily schedule were associated with prohibitive toxicity. We have explored a weekly schedule and found a marked increase in the therapeutic index of methyl-GAG. Responses were observed in a wide variety of solid tumors, particularly types known to be unresponsive to chemotherapy. Tox-

TABLE 2.—Response by diagnosis

Diagnosis	No. of evaluable patients	No. of patients with complete or partial response	Duration of response (mos)	No. of patients with—			
				Complete response	Partial response	Improvement	No response
Renal (13)*	5	1	5		1	2	2
Colon (27)	14	3	5,5,7+		3		11
Pancreas (7)	5	2	7+,8+		2		3
Lung (7) (non-small cell)	6	2	2,7+		2		4
Esophagus (4)	2	1	3		1		1
Bladder (4)	3	1	1	1			2
Adenocarcinoma (4) (unknown primary)	3	1	2	1		1	1
Breast (18)	12					2	10
Lymphoma (6)	2						2
Prostate (11)	6					1	5
Head and neck (2)	2						2
Hepatoma (2)	1						1
Melanoma (1)	1					1	
Leukemia (1)†	1					1	
Ewing's sarcoma (1)	1						1
Oat cell (1)	1					1	
Totals	65	11		2	9	9	45

*Nos. in parentheses = No. of patients entered in the study.
†Patient received 1 g/m²/wk for 3 wks.

icity was for the most part unpredictable, and seemed to be cumulative rather than dose-related. This may be interpreted in part by very limited pharmacologic data in man which suggest prolonged retention and delayed excretion, primarily of intact drug: only 60% of an administered iv dose was excreted in the urine and < 20% was excreted in the feces over a 3-week period (5). A similar pattern was obtained in dogs and monkeys after iv administration of the drug (10). In the dog, less than one half of a dose of 400 mg/m² was excreted over 96 hours, but > 75% had been recovered in the urine by 7 days.

Although the mechanism of antitumor action of methyl-GAG is still not elucidated, it may be unique for chemotherapeutic agents and related to polyamine biosynthesis. Physiologically achievable effects of methyl-GAG may be related to inhibition of the enzyme *S*-adenosylmethionine decarboxylase which catalyzes synthesis of spermidine (11). However, this enzyme is also stabilized by methyl-GAG (12). At concentrations as low as 0.5 μg/ml, methyl-GAG is a potent inhibitor of spermidine biosynthesis (13,14). At concentrations of 2–8 μM (0.6–2.4 μg/ml), methyl-GAG appears to act selectively on polyamine synthesis. At concentrations as high as ≥ 100 μM (30 μg/mg), the drug has other direct toxic effects, including inhibition of protein synthesis (14) and complete inhibition of mitochondrial respiration (15).

There is a growing body of evidence to suggest that spermidine, in particular, is of importance in the initiation of DNA synthesis, possibly through stabilization of the DNA polymerase-helix complex (16). Several studies have shown that methyl-GAG-mediated depression of DNA synthesis is associated with spermidine depletion and putrescine accumulation, and that spermidine in equimolar quantities can reverse this effect in living cells (17,18).

A second area in which polyamines play a major role is in RNA synthesis, especially that of transfer (t) RNA (19). The methylation of tRNA may be directly stimulated by polyamines (20), a finding of particular interest in view of the reports that neoplastic tissue differs from normal tissue with respect to the extent of methylated tRNA (21). Here, too, spermine (and spermidine) appears to play a critical role (22).

Polyamine accumulation appears to be a necessary concomitant of DNA synthesis at an optimal rate, in both normal and neoplastic tissues. Thus, the toxicity of methyl-GAG observed in tissues with rapid turnover (skin, gastrointestinal mucosa, and bone marrow) may be directly related to inhibition of polyamine biosynthesis. There is, however, strong evidence that: (*a*) polyamines are excreted in excess in the majority of cancer patients (23); (*b*) polyamines, especially spermidine, are released from tumor cells during and after effective chemotherapy, with an initial peak in excretion and in serum levels and subsequent drop toward normal values (24); and (*c*) chemotherapy, which produces only bone marrow (or other normal tissue) toxicity and is without antitumor effectiveness, does not produce a significant increase in polyamine excretion (24). The latter observation would suggest either that cancer cells have much higher levels

TABLE 3.—Toxic effects in 90 patients

Toxic effect	No. of patients (%)
Mucositis	17 (19)
Erythema	3
Ulcers, able to eat	7
Ulcers, unable to eat	6
Drug-related death	1
Nausea and vomiting	14 (16)
Nausea, no vomiting	5
Vomiting ≤ 6 times/day	5
Vomiting ≥ 7 times/day	3
Hospitalization secondary to vomiting	1
Anorexia	7 (8)
Diarrhea	3 (3)
3–4 stools/day, no dehydration	1
> 4 liquid stools/day, iv hydration required	1
Bloody diarrhea	1
Vasculitis	2 (2)
Myopathy	3 (3)
Balanitis	4 (4)
Urethritis	3 (3)
Skin rash	2 (2)
Hematologic	10 (11)
Anemia	
Hemoglobin level (g/100 ml)	
7.0–8.9	1
5.0–6.9	2
Platelet count (25,000–50,000/mm^3)	2
Granulocyte count	
< 1500/mm^3	2
< 1000/mm^3	3

TABLE 4.—Cumulative toxicity

No. of courses of methyl-GAG	No. of patients	No. of patients with toxic effects (%)
1	13	1 (8)
2	25	7 (28)
3	15	6 (40)
≥ 4	56	28 (50)

of polyamines than normal cells, even those with higher rates of DNA synthesis, or that therapy which is effective produces rather specific effects on polyamine synthesis in cancer cells.

The possibly unique mechanism of action and low incidence of myelosuppression with methyl-GAG are promising leads for combination chemotherapy, if the optimum dose and schedule can be delineated. Further human pharmacologic data are needed to improve the therapeutic index of this compound. Encouraging results in refractory colon, renal, and pancreatic cancer warrant further clinical trials.

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Pharmacokinetic Model for 2-Amino-1,3,4-thiadiazole in Mouse, Dog, and Monkey [1]

Franklin G. King[2,3] **and Robert L. Dedrick**[2,*]

SUMMARY

A mathematic model has been developed to describe the time course of 2-amino-1,3,4-thiadiazole and its metabolites in the serum and tissues of mice, dogs, and monkeys. A flow-limited physiologic model has been used. The rate of metabolism in mice is described as a linear process; metabolism in dogs and monkeys is kinetically saturable. The urinary excretion in mice, dogs, and monkeys is described as a linear process; however, the kidney clearance in monkeys may decrease at high plasma concentrations. The model predictions are compared with published data. The kidney clearances of 2-amino-1,3,4-thiadiazole and its metabolites exhibit approximately a 0.7-power dependence on body weight when the mouse is compared with the larger species.

[Cancer Treat Rep 63:1939–1947, 1979]

The anticancer activity of 2-amino-1,3,4-thiadiazole (ATDA) has been known for some time. Oleson et al (1) reported that ATDA retarded the growth of a melanoma, a glioblastoma, and a lymphosarcoma in mice. The antileukemic action of ATDA was reported by Ciotti et al (2). Ciotti also reported that the antileukemic activity could be reversed by nicotinamide. The mechanism of action of ATDA was reported by Nelson et al (3). Two derivatives of ATDA were found to inhibit the action of inosine-5-phosphate (IMP) dehydrogenase in leukemia cells.

Lu et al (4) reported preliminary studies on the distribution and metabolism of ATDA in beagle dogs. El Dareer et al (5,6) conducted an extensive experimental study to determine the distribution and metabolism of ATDA in mice, dogs, and monkeys.

We report the development of a physiologic pharmacokinetic model to describe the kinetics of distribution, metabolism, and excretion of ATDA. Model simulations are compared with available published data.

MODEL DEVELOPMENT

The development of physiologically based models to describe pharmacokinetic behavior has been presented in a number of sources (7–9). Basically, the method differs from the classic compartmental approach in that the compartments have anatomic and physiologic significance. In addition, many of the model parameters can be independently obtained and verified. Figure 1 is a representation of a compartment which does not eliminate the drug. A drug enters a compartment at the arterial concentration. It is well-mixed in the extracellular space and leaves at the venous concentration. The average tissue concentration may differ from the venous concentration depending on the extent of penetration to the intracellular space.

To simplify the model and to eliminate the need for cell membrane permeabilities, it was assumed that ATDA

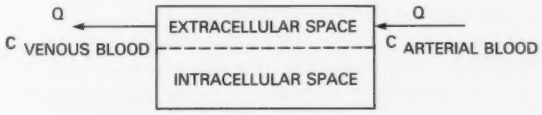

FIGURE 1.—Representation of a lumped compartment.

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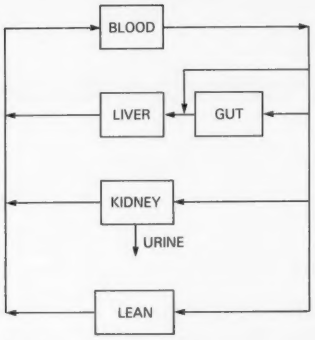

FIGURE 2.—Compartment model for ATDA pharmacokinetics.

transport is perfusion-limited. The flow-limited assumption means that tissue uptake is rapid compared with drug delivery in arterial blood so that the tissue concentration and venous concentration are in equilibrium. Plasma protein binding of ATDA was neglected because data (6) indicate that the drug is insignificantly bound (3%–7%) to plasma proteins. Linear binding in the tissues is accounted for by tissue/venous serum distribution ratios.

Since only 2% of the ATDA dose was eliminated in the feces of dogs and monkeys, and none was reported in mice (6), the fecal route of elimination was neglected. Urinary excretion of ATDA ranged from 20% to 80% in 24 hours, the remainder of the drug being eliminated as metabolite. The kidney was included as the primary site of drug elimination. A linear elimination model was used.

The metabolism of ATDA appears to be a complex biochemical process. The active form of ATDA reported by Nelson et al (3) is a phosphorylated specie that binds tightly to IMP dehydrogenase. This charged specie was formed in L1210 cells and would be expected to be trapped intracellularly. In an in vivo experiment, Nelson et al (3) reported that the concentration of phosphorylated metabolite in L1210 cells was 0.03 μg/ml following a dose of 5 mg/kg. If the data of El Dareer et al (6) are scaled to the same dose, this concentration represents 5%–10% of the total metabolite levels found in other tissues. El Dareer et al (6) found three metabolites of ATDA which appeared in the serum and urine of mice, dogs, and monkeys. It is probable that none of these metabolites was the phosphorylated form. Although three metabolites were found (6), the data were reported as total metabolite concentration. We have also lumped these metabolites for purposes of analysis.

Pharmacokinetically, the intrinsic metabolic clearance of ATDA is much lower than blood flow to major probable sites of metabolism such as the liver. Thus, since the metabolic rate is small, the site of metabolism is less important for ATDA than for a drug like cytosine arabinoside which is rapidly metabolized (10). Since the site of metabolism of ATDA is unknown, we have arbitrarily placed the metabolism in the liver. Computer simulation has shown, however, that the time course of ATDA and its metabolites was not affected by placing the metabolism in the liver or the blood, or distributing the metabolism among the blood, liver, and kidney. A saturable kinetic model was used to describe the metabolism in dogs and monkeys because a zero order elimination was reported. A linear model was used for mice.

The kinetic model used here can be extended when biochemical reactions and sites are more completely known. A similar extension of a pharmacokinetic model was reported by Morrison et al (11) for cytosine arabinoside and its active phosphorylated metabolite when data became available.

Data indicate that the volume of distribution was on the order of 80%–85% of body weight for each species. In order to accurately describe the ATDA behavior and keep the model as simple as possible, a lean compartment was used which consisted of muscle, skin, and marrow tissues.

The flow diagram for the model is shown in figure 2. The resulting model consists of a set of mass balance differential equations which define the concentration of ATDA and metabolite in each compartment. The equations were solved numerically to predict the time course for the concentration of ATDA and metabolite in each tissue and cumulative excretion in the urine. The differential equations used are summarized in the appendix.

The ip dose given to mice was approximated as an exponential input to the liver as has been previously reported (10). The time constant parameter which accounts for the rate of drug absorption from the peritoneal cavity is the fluid volume in the peritoneal cavity divided by a permeability area product (12). The time constant for the absorption of ATDA was found by computer fitting to be 15 minutes. This result indicates that most of the drug is absorbed in < 1 hour.

DETERMINATION OF MODEL PARAMETERS

A large number of parameters are needed in a physiologic model. Anatomic tissue sizes and perfusion rates can usually be estimated from various sources (9,10,13). Table 1 summarizes the volumes and perfusion rates for the various compartments (10). The other model parameters were estimated from the available experimental data for ATDA.

The tissue/venous serum distribution ratios were estimated using the serum data of El Dareer et al (6). Venous serum concentrations were estimated using pooled serum from the axillary region of the mouse and serum from the cephalic, bronchial, and saphenous veins of dogs and monkeys. Distribution data (6) for mice were

TABLE 1.—Physiologic parameters (*from* ref 10)

	Mouse	Dog	Monkey
	Compartment volumes (ml)		
Blood	1.90	670	367
Liver	1.48	250	135
Kidney	0.39	50	30
Gut	1.70	400	230
Lean*	16.50	6870	3435
Whole animal	25 g	10 kg	5 kg
	Compartment flow rates (ml/min)		
Blood	4.85	837	400
Liver†	0.35	54	28
Kidney	1.58	217	123
Gut	1.70	217	125
Lean	1.32	350	124

*Lean includes skin and marrow. Perfusion rates of skin and muscle are assumed to be equal.

†Hepatic artery flow rate.

available at several time points and were correlated as in figure 3. Implicit in figure 3 is the flow-limited assumption that the tissue concentration and venous serum concentration are in equilibrium. The tissue/venous serum ratios of ATDA and metabolite appear to be independent of concentration, since the lines in figure 3 have a slope of unity. Data for the dog and monkey were available at only a single time point. Initial estimates for the distribution ratios were then adjusted slightly (no more than 10%) to give the best match between the computer simulation and the tissue concentration data.

The kidney clearances of ATDA and metabolite were estimated by dividing the amount of each species appearing in the urine by the area under a curve of serum

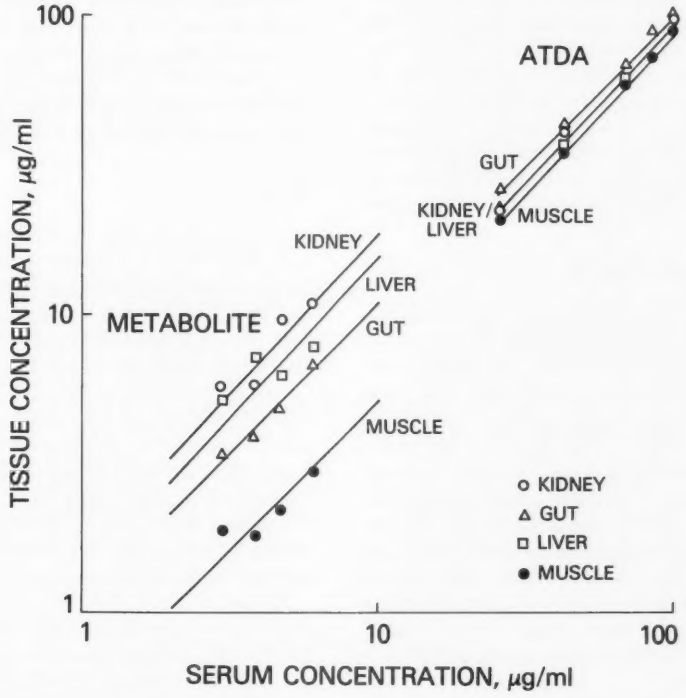

FIGURE 3.—Tissue/serum distribution of ATDA and metabolite in mice.

TABLE 2.—Pharmacokinetic parameters

	Tissue/serum distribution ratios					
	Mouse		Dog		Monkey	
	ATDA	Metabolite	ATDA	Metabolite	ATDA	Metabolite
Blood	1.0	1.0	1.0	1.0	1.0	1.0
Liver	0.9	1.3	0.9	2.8	0.6	1.8
Gut	1.0	1.1	1.0	0.4	0.4	1.3
Kidney	0.9	2.0	1.6	2.0	0.6	3.6
Lean	0.9	0.5	1.0	0.2	0.9	0.3

	Kinetic parameters		
	Mouse	Dog	Monkey
Kidney clearances (ml/min)			
ATDA	0.066	8.8	1.5
Metabolites	0.215	18.3	4.6
GFR (estimated)	0.300	40.0	18.0
Metabolism			
V_{max} (μg/min)		19.2	6.3
K_m (μg/ml)		0.2	0.5
k or V_{max}/K_m (ml/min)	0.017	95.0	12.5

concentration over the given time period. Order of magnitude estimates of the metabolism rates were estimated as the difference between the kidney clearance and a whole-body clearance obtained by dividing the dose by the area under the serum concentration versus time curve. These estimates of kidney clearance and liver metabolism parameters were adjusted automatically by means of a computer search with the objective of minimizing the least square of the error between the model predictions and the experimental data for the serum concentration versus time curve. Table 2 summarizes the model parameters for ATDA and metabolite for the three species.

RESULTS AND DISCUSSION

The physiologic model was used to predict the time course of ATDA and metabolite concentration in serum and several tissues in the mouse, dog, and monkey. The model was also used to predict urinary excretion. In all cases it was assumed that serum concentration was equal to blood concentration because the drug does not bind to protein significantly and appears to distribute to the intracellular space of tissue. The model predictions are compared with the experimental data of El Dareer et al (6).

Model predictions of serum concentration of ATDA and metabolite are compared with the experimental data of El Dareer et al (6) in figures 4 and 5 for the mouse and dog at doses of 100 and 3 mg/kg respectively. Figure 6 presents comparisons for three dose levels of ATDA in the monkey. Model predictions of concentrations in several tissues at 4 hours are compared with data in table 3.

The simulation underpredicts serum levels of ATDA metabolite at early times in the mouse and dog. This may suggest that transport of metabolite is not rigorously flow-limited in all tissues so that volume of distribution could increase with time. This hypothesis is supported by muscle concentration data at 0.5 hour in the mouse. This time point was excluded from figure 3. The apparent distribution ratio for muscle at 0.5 hour was significantly less than the equilibrium value of 0.5.

Mice

The model predictions of urinary excretion of ATDA and its metabolite in mice are compared with the data in figure 7. Similar results were obtained for the dog and monkey. While the model simulates tissue and serum data very well, it tends to overpredict urinary excretion. This discrepancy may result from incomplete experimental recovery. Material balances indicated that between 84% and 94% of the drug could be accounted for at any time in the tissues modeled or in the urine. Since the model must account for all of the dose, the model was allowed to overpredict urinary excretion in order to match serum and tissue concentrations. Better formal simulation could easily have been obtained by normalizing concentrations with respect to recovery.

The metabolism of ATDA in mice was modeled as a linear process occurring in the liver. The time course of ATDA elimination from serum, shown in figure 4, forms a straight line which is characteristic of a first-order process. This evidence indicates that a dose of 100 mg/kg does not saturate the metabolic or urinary excretion path-

TABLE 3.—Tissue distribution of ATDA and metabolite (4 hrs following dose)

	ATDA concentration (μg/ml)		Metabolite concentration (μg/ml)	
	Data	Model	Data	Model
	Mouse (following 100-mg/kg ip dose)			
Liver	37 ± 2	39	7.1 ± 0.4	6.5
Gut	44 ± 5	47	3.8 ± 0.4	5.1
Lean	35 ± 3	39	1.8 ± 0.2	2.1
Kidney	42 ± 2	40	5.7 ± 0.3	7.9
	Dog (following 3.0-mg/kg iv dose)			
Liver	2.03	2.05	1.88	2.34
Gut	2.30	2.34	0.26	0.27
Lean	2.52	2.30	0.11	0.12
Kidney	3.66	3.58	1.29	1.42
	Monkey (following 3.0-mg/kg iv dose)			
Gut	1.02	1.16	0.78	0.69
Kidney	1.84	1.96	2.17	1.84
Lean	2.40	3.03	0.07	0.13
Liver	1.85	1.96	1.08	1.01

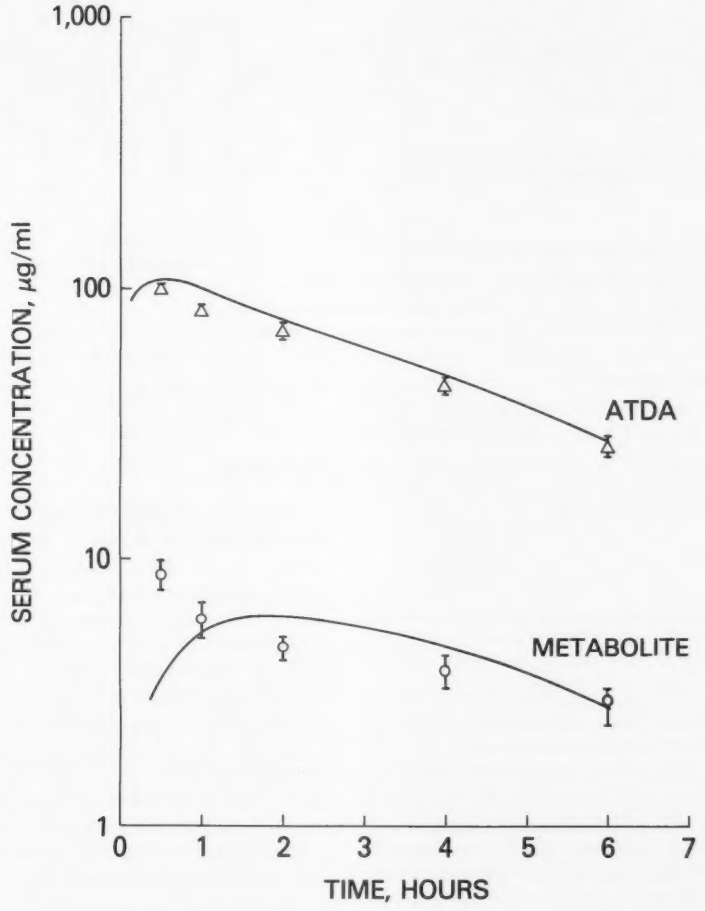

FIGURE 4.—Model prediction of mouse serum concentration compared with experimental data (following 100-mg/kg ip dose).

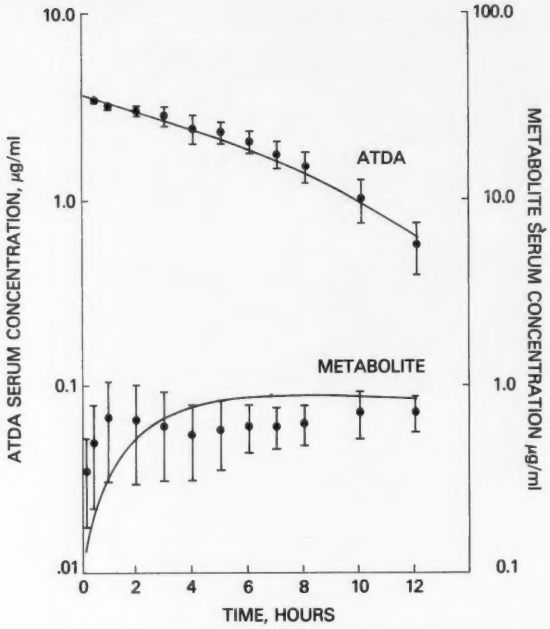

FIGURE 5.—Model prediction of dog serum concentration compared with experimental data (following 3-mg/kg iv dose).

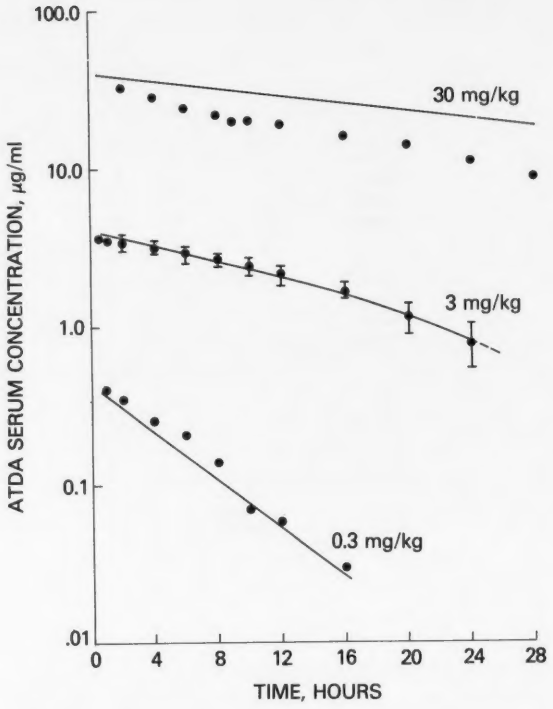

FIGURE 6.—Model prediction of monkey serum concentration compared with experimental data.

ways. The first-order rate constant for metabolism is 0.017 ml/minute, and it does not show any concentration dependence. The kidney clearances for the mouse are 0.066 ml/minute for ATDA and 0.215 ml/minute for metabolite. The results indicate that kidney clearance is about 4 times the metabolic clearance. In addition, the kidney clearance appears constant over the concentration range studied, even though it is only 22% of the estimated glomerular filtration rate (GFR). Since ATDA is not significantly bound to plasma proteins, considerable reabsorption must occur in the kidney.

Dogs

Experimental data have shown that the elimination of ATDA from dog serum is a saturable process following an iv dose of 3 mg/kg. This is shown by the non-linear curve for ATDA in figure 5. This behavior may be described by considering only the metabolism to be saturable. A computer-fitted Michaelis constant of 0.2 μg/ml was obtained. Since the serum concentration remained in the range of 0.4–3.5 μg/ml, the metabolism was almost saturated over the entire time course. The kidney clearance of ATDA was found to be 8.8 ml/minute. The ratio of kidney clearance to estimated GFR for the dog is about the same as in mice, providing some support for modeling the urinary excretion as a linear process. The saturated metabolic pathway initially has a clearance of 5.2 ml/minute. This increases to 32 ml/minute as the serum concentration decreases and would reach 95 ml/minute at low concentrations (as inferred from the model).

Monkeys

Experimental data for the time course of ATDA serum concentration as shown in figure 6 indicate that elimination of ATDA from monkey serum is a linear process at the lowest dose level and is non-linear at the two higher doses. The Michaelis constant for metabolism was found to be 0.5 μg/ml, which is similar to that observed in the dog. The model predicted the data very closely for the two lower doses, as shown in figure 6. The model overpredicted the serum concentration at the 30-mg/kg dose, indicating that the saturable metabolism/linear kidney clearance model could not account for the rate of clearance. One explanation, as first suggested by El Dareer et al (6), is that the relative re-absorption in the kidney may decrease at very high concentrations. While a more complex kidney model may be required, it does not appear to be justified here since the experimental data exist for only one animal of one species in which the kidney

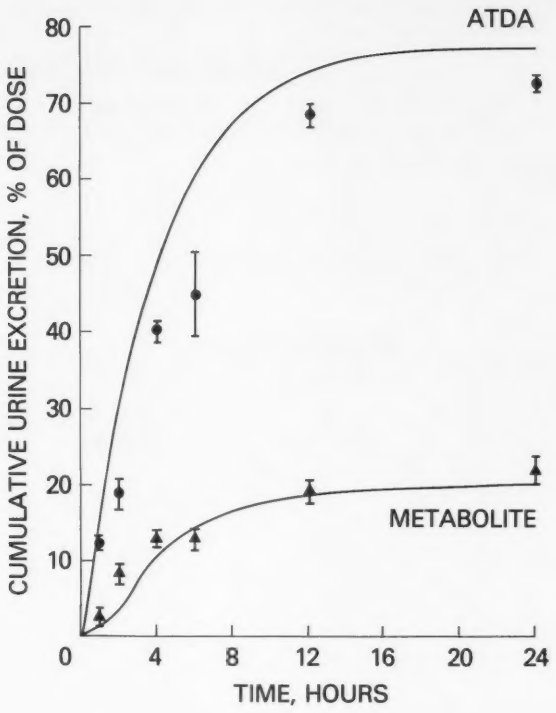

FIGURE 7.—Model prediction of cumulative urine excretion in mice (following 100-mg/kg ip dose).

clearance is not constant. The monkey had the least ability to excrete ATDA through the kidneys based on the observation that the clearance of 1.5 ml/minute was about 8% of the estimated GFR of 18 ml/minute. The dog and mouse clearances were about 22% of the GFR and both were constant.

We have attempted to correlate the metabolic and kidney clearances of ATDA and metabolite among the three species by plotting the clearance versus body weight on log-log paper (fig 8). A variety of physiologic functions can be correlated in this way (13). The slope of the lines for the kidney clearances is 0.70, which is similar to the value of 0.77 previously obtained for inulin. The slope of the curve for metabolic clearance is 1.29. This is a significant interspecies difference which could not be obtained from existing correlations. The difference indicates that kidney clearance dominates the excretion pathway in the mouse, whereas the metabolic pathway is greater for dogs and monkeys. Predictability of metabolism to humans would be uncertain.

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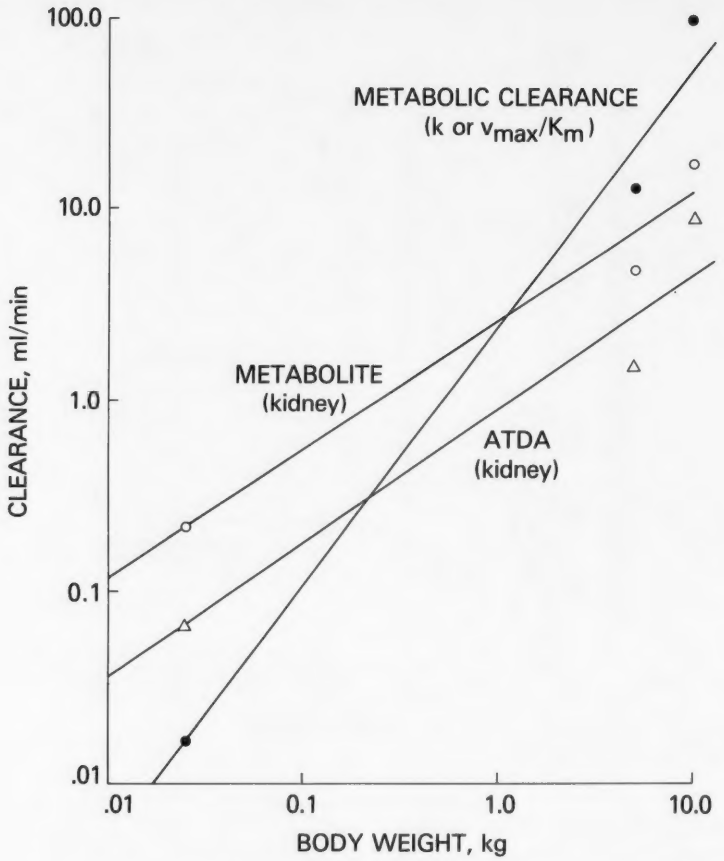

FIGURE 8.—Kidney and metabolic clearance of ATDA and metabolite for mice, dog, and monkey.

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APPENDIX.—Equations for the pharmacokinetic model. V = tissue volume (ml), C = concentration (μg/ml), t = time (mins), R = equilibrium tissue/serum ratio, D(t) = dose injection function (μg/min) (pulse for iv, exponential in liver compartment for ip), Q = blood flow rate (ml/min), M = rate of metabolism (μg/min), V_{max} = maximum rate of metabolism (μg/min), K_m = Michaelis constant (μg/ml), k_K = kidney clearance (ml/min). Subscripts S, L, G, K, and Le refer to the serum, liver, gut, kidney, and lean compartments respectively, while the prime designates metabolite.

SERUM

ATDA: $$V_S \frac{dC_S}{dt} = D(t) + (Q_L + Q_G)\frac{C_L}{R_L} + Q_K \frac{C_K}{R_K} + Q_{Le}\frac{C_{Le}}{R_{Le}} - Q_S C_S$$

METABOLITE: $$V_S \frac{dC'_S}{dt} = (Q_L + Q_G)\frac{C'_L}{R'_L} + Q_K \frac{C'_K}{R'_K} + Q_{Le}\frac{C'_{Le}}{R'_{Le}} - Q_S C'_S$$

LIVER

ATDA: $$V_L \frac{dC_L}{dt} = Q_L C_S + Q_G \frac{C_G}{R_G} - (Q_L + Q_G)\frac{C_L}{R_L} - M + D(t)$$

METABOLITE: $$V_L \frac{dC'_L}{dt} = Q_L C'_S + Q_G \frac{C'_G}{R'_G} - (Q_L + Q_G)\frac{C'_L}{R'_L} + M$$

for linear metabolism, $$M = k_m \frac{C_L}{R_L}$$

for saturable metabolism, $$M = \frac{V_{max}}{K_M + \frac{C_L}{R_L}}\frac{C_L}{R_L}$$

KIDNEY

ATDA: $$V_K \frac{dC_K}{dt} = Q_K C_S - Q_K \frac{C_K}{R_K} - k_K C_S$$

METABOLITE: $$V_K \frac{dC'_K}{dt} = Q_K\left(C'_S - \frac{C'_K}{R'_K}\right) - k'_K C'_S$$

GUT/LEAN

Same as kidney without the elimination term.

Treatment of Acute Lymphoblastic Leukemia With 2′-Deoxycoformycin: Clinical and Biochemical Consequences of Adenosine Deaminase Inhibition [1,2]

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SUMMARY

Recent studies have documented that deoxyadenosine, in the presence of an adenosine deaminase inhibitor, is markedly toxic to cultured T-lymphoblasts derived from patients with acute lymphoblastic leukemia. The molecular basis of cytotoxicity is felt to be an accumulation of the metabolite deoxyadenosine triphosphate (dATP), resulting in inhibition of DNA synthesis. We have treated a 23-year-old patient with acute lymphoblastic leukemia with 2′-deoxycoformycin, a potent inhibitor of adenosine deaminase, and have monitored leukemic cell dATP levels as a parameter of therapeutic response. The patient received 2′-deoxycoformycin, 18.25 mg (0.25 mg/kg/day), iv daily for 5 days during 2 consecutive weeks without apparent toxicity. There was an immediate fall of > 98% in adenosine deaminase activity in circulating wbcs, rbcs, and serum which persisted for 12 days, the duration of the study. Leukemic marrow cell adenosine deaminase activity was also inhibited. In vitro studies before treatment revealed an accumulation of 49 pmols of dATP/10^6 cells following a 1-hour incubation of leukemic cells with 5 μM 2′-deoxycoformycin and 50 μM deoxyadenosine. After 5 days of treatment, the level of dATP in leukemic cells was only 9 pmols/10^6 cells, but a further incubation of cells for 1 hour with 50 μM deoxyadenosine alone increased dATP levels to 35 pmols/10^6 cells. These data suggest that accumulation of dATP by lymphoblasts in vivo may have been limited by inavailability of deoxyadenosine. Lymphoblast proliferation was not inhibited by this course of therapy. Measurements of dATP accumulation by leukemic cells in vitro and in vivo may be helpful in predicting the clinical response to treatment with 2′-deoxycoformycin.

[Cancer Treat Rep 63:1949–1952, 1979]

Deficiency of the enzyme adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4; ADA) is associated with severe combined immunodeficiency disease characterized by thymic involution, variable B-lymphocyte dysfunction, and severe T-lymphocyte dysfunction (1,2). ADA catalyzes the deamination of adenosine and deoxyadenosine (AdR) to inosine and deoxyinosine respectively, and it has been proposed that increased levels of

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AdR lead to the selective accumulation of deoxyadenosine triphosphate (dATP) by lymphoid cells in this disorder (3). dATP is a potent inhibitor of the enzyme ribonucleotide reductase (4) and is presumed to effect cytotoxicity by depleting cells of other deoxyribonucleotides required for DNA synthesis (5,6).

Recent in vitro studies have demonstrated that AdR, in the presence of an ADA inhibitor, is markedly toxic to cultured T-lymphoblasts derived from patients with acute lymphoblastic leukemia (ALL). Cytotoxicity correlated with the ability of these cells to accumulate dATP (7,8). On the basis of these studies, we have treated a patient with ALL refractory to standard therapy with 2′-deoxycoformycin (2′-DCF), a potent inhibitor of ADA. Although the patient failed to respond to this agent, we have gained further insight into the clinical and biochemical effects of ADA inhibition.

PATIENT AND METHODS

Case Report

A 23-year-old male presented in September 1976 with fatigue, lymphadenopathy, and splenomegaly. The peripheral blood count revealed a hemoglobin level of 6.8 g/dl, a wbc count of 134,000/mm^3 with 96% lymphoblasts, and a platelet count of 58,000/mm^3. The bone marrow was hypercellular with 98% lymphoblasts of the L2 variety; an occasional megakaryocyte was seen. The blast cells did not form E or EAC rosettes and lacked surface immunoglobulins, and thus could not be classified as either T- or B-cells. Terminal deoxynucleotidyl transferase was present as shown by a fluorescent antibody stain. The leukemic lymphoblasts developed E-rosetting capacity after 48 hours of culture at 37°C in RPMI 1640 medium with 10% fetal calf serum. Intensive induction chemotherapy with vincristine, prednisone, Adriamycin, and cyclophosphamide was initiated. The patient did not enter remission on this regimen or on subsequent protocols which included bleomycin, L-asparaginase, methotrexate, 6-mercaptopurine, and/or prednisone. He had received no chemotherapy for 4 weeks prior to initiation of this study, at which time his wbc count was 3200/mm^3 with 48% polymorphonuclear neutrophils, 49% mature lymphocytes, and no blast cells. The bone marrow, however, remained hypercellular with > 95% blast cells. Serum immunoglobulins were within the normal range. *Candida*, mumps, intermediate-strength purified protein derivative, and streptokinase-streptodornase skin tests were negative; a phytohemagglutinin skin test was positive (8 mm). There was no stimulation of peripheral blood lymphocytes by phytohemagglutinin, concanavalin A, or pokeweed mitogen. Allopurinol therapy (300 mg/day) was started prior to the study.

Preparation and Administration of Drug

2′-DCF was obtained from the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD) and was found to be sterile and free of pyrogens. Each dose of 0.25 mg/kg (18.25 mg) was dissolved in 20 ml of Ringer's lactate with 44.6 mEq/liter of $NaHCO_3$ (pH 8.0) and was administered iv over 10 minutes. The patient was treated once daily for 5 days during 2 consecutive weeks.

Approval for administration of 2′-DCF was obtained from the Human Subjects Review Committee of Ohio State University and from the Food and Drug Administration. Informed written consent was obtained from the patient prior to initiation of the study.

Adenosine Deaminase Assay

ADA was assayed by an adaptation of the micromethod of Kizaki and Sakurada (9), measuring the conversion of 8-^{14}C-adenosine to inosine. Enzyme activity is expressed as nmols of inosine formed/hour per 10^6 cells for wbcs or per mg of protein for rbcs or serum.

Measurement of Intracellular Deoxyadenosine Triphosphate Levels

Deoxyribonucleotides were extracted from lymphoblast pellets with 1 ml of cold 60% aqueous methanol. Samples were stored at –20°C overnight and evaporated to dryness. Residues were suspended in 50 µl of distilled water and assayed for dATP by an adaptation of the method of Solter and Handschumacher (10), as previously described (7). Deoxyribonucleotides were extracted from 1-ml pellets of washed rbcs by the addition of 1 ml of 2.0 M cold perchloric acid and neutralization to pH 6.8 with 5.0 M KOH. Extracts were lyophilized and suspended in 100 µl of distilled water for assay.

Assay of Adenosine or Deoxyadenosine

The concentrations of adenosine and AdR in plasma were determined by the method of Mollering and Bergmeyer (11).

RESULTS

The administration of 2′-DCF at a dose of 0.25 mg/kg/day was well-tolerated and was not associated with any changes in hepatic or renal function. Serum uric acid remained < 6 mg/100 ml throughout the study. Over the initial 5 days of treatment, the patient developed progressive leukopenia associated with an increase in peripheral lymphoblasts from 0 to 30% (fig 1). The bone marrow remained hypercellular with > 95% blast cells.

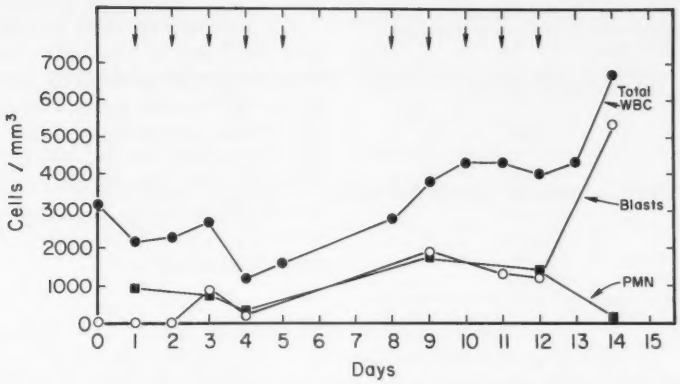

FIGURE 1.—Total wbcs (—●—●—), lymphoblasts (—○—○—), and polymorphonuclear neutrophils (—■—■—) during course of treatment with 2′-DCF. Days on which 2′-DCF was administered are marked by arrows.

After the second week, the wbc count had increased to 6700/mm^3 with 80% lymphoblasts, and therapy was discontinued. At this time, serum immunoglobulins were still within the normal range, but the positive skin test to PHA became negative.

Sequential measurements of ADA activity in buffy coat cells, mononuclear cells, peripheral blood hemolysate, and serum showed a complete inhibition of activity within 1 hour of drug administration, which was sustained over the course of therapy (table 1). ADA activity in bone marrow cells was similarly inhibited when measured after 6 days. Despite this inhibition of ADA activity, plasma adenosine and AdR levels remained < 1 μM, which was the lower limit of detectability in our assay. Serial measurements of erythrocyte dATP levels demonstrated a rise to 2250 pmols/ml of packed rbcs by the fifth day of therapy (table 2). dATP levels in leukemic cells rose to only 9 pmols/10^6 cells following 5 days of therapy (table 3).

Leukemic lymphoblasts were incubated with 2′-DCF and/or AdR before and after the first week of therapy to determine their ability to accumulate dATP in vitro (table 3). Baseline studies indicated that dATP was not detectable in 10^6 lymphoblasts incubated for 1 hour alone or in the presence of 2′-DCF. In pretreatment lymphoblasts in the presence of both 50 μM AdR and 5 μM 2′-DCF for 1 hour, however, dATP levels rose to 49 pmols/10^6 cells. Following the course of therapy with 2′-DCF, incubation of leukemic cells with 50 μM AdR alone in the absence of additional 2′-DCF increased the dATP levels nearly fourfold from 9 to 35 pmols/10^6 cells.

DISCUSSION

Although the exact mechanism by which ADA deficiency results in lymphocytotoxicity is not known, recent work strongly supports the concept that elevated circulating AdR is selectively phosphorylated to dATP in lymphoid cells, resulting in inhibition of DNA synthesis and cell death. The use of 2′-DCF in the treatment of refractory ALL thus offers a unique opportunity to mon-

TABLE 1.—Effect of 2′-DCF on ADA activity

Specimen	ADA activity*						
	Pretreatment	Posttreatment					
		1 hr	2 hrs	12 hrs	2 days	4 days	6 days	12 days
Bone marrow cells	289.0						6.2	
Buffy coat cells	119.0	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Mononuclear cells	118.0	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Rbc hemolysate	56.0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Serum	4.5	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

*Expressed as nmols/hr/10^6 cells for bone marrow cells, buffy coat cells, and mononuclear cells, and as nmols/hr/mg of protein for rbc hemolysate and serum.

TABLE 2.—Effect of therapy with 2′-DCF on erythrocyte dATP levels

Time after start of therapy	dATP levels (pmols/ml of packed rbcs)
0	< 20
1 hr	< 20
4 hrs	< 20
3 days	< 20
4 days	< 20
5 days	2250

TABLE 3.—Accumulation of dATP by leukemic cells before and after therapy with 2′-DCF

Additives*	dATP ($pmols/10^6$ cells)	
	Before therapy	Day 6
None	< 5	9
50 μM AdR	8	35
1 μM 2′-DCF	< 5	8
5 μM 2′-DCF	< 5	10
50 μM AdR + 1 μM 2′-DCF	31	48
50 μM AdR + 5 μM 2′-DCF	49	52

*Cells were incubated at a concentration of 10^6/ml in 1 ml of RPMI 1640 medium + 10% heat-activated horse serum for 1 hr in the presence of the additives indicated. dATP was extracted from the cell pellet with 60% aqueous methanol.

itor the biochemical as well as the clinical consequences of ADA inhibition in a malignant lymphoproliferative disorder.

Administration of 2′-DCF to a single patient with ALL was well-tolerated and resulted in inhibition of ADA activity in peripheral blood and leukemic marrow cells, as has recently been reported in a group of seven patients with ALL from England (12). The lack of clinical response in our patient may well be attributable to insufficient dATP accumulation by the leukemic cells. Although the level of dATP required for inhibition of DNA synthesis has not been established, the posttreatment dATP value of 9 pmols/10^6 leukemic cells is clearly less than the normal dATP levels of cultured T-lymphoblasts (7). The fourfold increase in leukemic cell dATP in the presence of additional AdR in vitro suggests that dATP accumulation in vivo may have been limited by inavailability of AdR. This interpretation is supported by the dATP level in rbcs of only 2500 pmols/ml on Day 5 of therapy, a value approximately 1% of that found in erythrocytes from ADA-deficient individuals (13,14).

It is possible that more prolonged inhibition of ADA by 2′-DCF may be required to increase AdR concentrations to lymphotoxic levels. Alternatively, exogenous AdR could be added to the therapeutic regimen. Because our studies represent the results of 2′-DCF therapy in only one patient, we feel that further therapeutic trials with this drug are warranted. Serial dATP measurements may be useful both in predicting clinical responsiveness and in assessing the need for AdR supplementation.

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Phase I Study of Weekly Maytansine Given by Iv Bolus or 24-Hour Infusion [1,2]

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SUMMARY

Maytansine was administered weekly by iv bolus or by 24-hour infusion to 71 patients with leukemia, lymphoma, or carcinoma. The most prominent toxicity was neurologic, consisting of a variable combination of a peripheral, predominantly sensory polyneuropathy and a generalized psychomotor depression of apparently central origin. Neurologic toxicity occurred with unacceptable frequency and severity when a cumulative dose of 6 mg/m^2 was exceeded at weekly doses ranging from 0.4 to 1.3 mg/m^2 by iv bolus. Even one or two doses at 1.4–1.6 mg/m^2/week produced severe neurologic toxicity. Nerve conduction velocities and electromyography were not clearly correlated with clinical neuropathy. Gastrointestinal toxicity, manifested by nausea, vomiting, diarrhea, and abdominal cramps, was frequent at dose levels of $\geq$ 1.1 mg/m^2/week. A few patients experienced severe hepatotoxic effects and myelosuppression, especially after the 24-hour infusion schedule. Paravenous infiltration produced local cellulitis or phlebitis. Skin rash and fever were uncommon. One patient each with carcinoma of the lung, thymoma, and diffuse histiocytic lymphoma responded with > 50% regression of tumor. The recommended dose for phase II trials is 1 mg/m^2/week by iv bolus for a total of six courses. Careful neurologic assessment is mandatory above this total dose level. Because of the lack of significant myelosuppression from this drug, further studies using maytansine, particularly in combination with other drugs in induction programs, are warranted.

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Maytansine is an ansa macrolide originally isolated in 1972 by Kupchan et al from the East African shrub *Maytenus ovatus* Loes, and later from the wood and bark of *Maytenus buchananii* (1,2). Its antitumor activity was experimentally demonstrated both in vitro and in vivo at extremely low dose levels (3). It is most effective against P388 leukemia in mice, and has shown cross resistance with vincristine in that system (3). Maytansine's principal mechanism of action seems to be a stathmokinetic effect, like the vinca alkaloids (4,5), but it seems to offer several advantages over vincristine. It binds more avidly to rat brain tubulin, both to a binding site common for it with vincristine and to an additional specific site (5). It is 100 times more potent than vincristine in inhibiting mitosis in sea urchin eggs and 15 times more potent in Chinese hamster ovary cells in culture (4). Maytansine also inhibits DNA synthesis (3).

Four phase I trials have already been completed. The recommended schedules for phase II trials have been 0.75 mg/m^2/day on Days 1, 3, and 5 every 4 weeks (6), 0.50 mg/m^2/day on Days 1, 3, and 5 every 2–3 weeks (7), 2–2.5

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mg/m² divided from Days 1 to 5 every 3 weeks (8), and 1.6 mg/m²/day every 3 weeks (9). In all of these trials, the drug was given by iv bolus or by infusion over 15–30 minutes. Acute gastrointestinal toxicity was dose-limiting, although neurologic and hepatic toxic effects were also mentioned. Few patients in these studies received more than one course, and no definition of cumulative toxicity was possible.

The purpose of the present study was to evaluate a weekly schedule of iv bolus administration of maytansine because of its similarities to vincristine and because of its lack of significant myelosuppression. We also decided to administer the drug by weekly 24-hour infusion in a subset of patients, since vincristine had shown activity with this schedule in acute leukemia (10). In addition, a weekly schedule was suitable to assess the cumulative toxicity of maytansine.

MATERIALS AND METHODS

Patient Selection

Seventy-one patients were entered in the study. There were 34 males and 37 females whose ages ranged from 6 to 84 years (median, 53 years). All of the patients had a histologically proven lymphoma, leukemia, or carcinoma (table 1) that was metastatic or locally invasive and for which conventional treatment by surgery, radiotherapy, or chemotherapy was unlikely to afford significant palliation. Fully informed consent was obtained from all patients or their parents or legal guardians. Most patients were ambulatory and were treated on an outpatient basis. Sixty-nine of 71 patients had received prior chemotherapy including vinca alkaloids (41 patients) and 51 patients had received prior radiotherapy.

Treatment Plan

Maytansine was supplied by the National Cancer Institute (Bethesda, MD) as a white lyophilized powder in vials containing 0.25 mg of maytansine and 100 mg of mannitol. Each vial was reconstituted with 5 ml of sterile water for injection immediately before use. Fifty-seven patients were treated in subsets by iv bolus weekly beginning at a dose of 0.4 mg/m². This initial dose corresponds to one third of the LD10 in rats (11,12), and early clinical trials showed that it was a safe starting dose in man. Subsets of three or more patients were treated at each dose level without dose escalation in the same patient. In the absence of prohibitive toxicity for a subset after at least 1 week, a new subset was started with a dose increment of 0.1 mg/m². This small increment was based on earlier clinical observations by others that toxic effects of maytansine such as uncontrollable diarrhea and dehydration were often unpredictable and difficult to quantify (7).

TABLE 1.—Tumor types of patients receiving phase I weekly maytansine

Tumor type	No. of patients receiving maytansine by—	
	Iv bolus	24-hr infusion
Breast	10	3
Acute myelocytic leukemia	8	4
Lung	11*	
Colon	6	
Sarcoma	3†	1‡
Acute lymphocytic leukemia	2	2
Hodgkin's disease	3	
Other lymphomas	2§	1¶
Thymoma	3	
Prostate	2	
Pleural mesothelioma	1	1
Multiple myeloma	1	1
Melanoma	1	
Gallbladder	1	
Adrenal		1
Kidney	1	
Ovary	1	
Glioblastoma	1	
Totals	57	14

* Includes 4 squamous cell, 3 adenocarcinoma, 3 small cell anaplastic, and 1 large cell anaplastic.

† Includes 1 each of spindle cell sarcoma, chondrosarcoma, and leiomyosarcoma.

‡ Synoviosarcoma.

§ Diffuse histiocytic and lymphosarcoma cell leukemia.

¶ Diffuse lymphocytic.

After assessment of toxicity by weekly bolus schedule, an additional subset of 14 patients received the drug by weekly continuous 24-hour infusion in 1000 ml of normal saline. The initial dose was 0.5 mg/m²/week and the increment was 0.2 mg/m²/week/patient. Toxicity was graded according to the Cancer and Leukemia Group B scoring system: 0 = none, 1 = mild, 2 = moderate, 3 = severe, and 4 = life-threatening.

Study Parameters

Physical examination, cbcs, and automated biochemical profile (SMA-6 and -12) were done in all patients prior to treatment and repeated weekly. Bone marrow aspirations were performed on the first and second day of the study in eight patients, and an additional aspiration was performed later in the study in three of these patients. Mitotic and labeling indices with tritiated thymidine were evaluated in bone marrow specimens of eight patients using a technique previously described (13). Sequential motor nerve conduction velocity determinations were performed in ten patients using surface recording and stimulating electrodes, and electromyograms were performed in eight patients with concentric needle elec-

TABLE 2.—No. of patients and dose escalation of weekly maytansine

Dose (mg/m^2)	Maytansine given by—			
	Iv bolus		24-hr infusion	
	No. of patients	No. of doses given	No. of patients	No. of doses given
0.4	4	3,4,7,10		
0.5	4	5,5,5,8	2	1,5
0.6	4	2,3,8,15*		
0.7	3	1,2,3	2	1,3
0.8	5	2,2,3,5,8		
0.9	3	3,4,8	2	1,3
1.0	6	2,4,4,6,6,7		
1.1	9	1,1,2,3,3,3,5,6,7	3	1,1,2
1.2	4	1,2,2,3		
1.3	7	1,1,1,2,2,5,9	3	1,2,5
1.4	2	2†,5		
1.5	5	1,2,3,4,5	2	2,2
1.6	1	2		
Totals	57		14	

*Reduced to 0.3 mg/m^2 after 12 doses.
†1st dose was 1.25 mg/m^2 and 2nd dose was 1.4 mg/m^2.

trodes. Therapeutic effects were evaluated according to standard criteria (14).

Patients were removed from the study when the maximally tolerated toxicity occurred and/or objective tumor progression was established.

RESULTS

A total of 256 courses of maytansine were given: 227 by iv bolus and 29 by 24-hour infusion (table 2). Three of the 71 patients treated were not fully evaluable because of early death from progressive disease (within 10 days). Of the 68 fully evaluable patients, 55 received maytansine by weekly iv bolus and 13 received maytansine by weekly 24-hour continuous infusion.

Gastrointestinal Toxicity

Twenty-five of the 57 patients receiving maytansine by weekly iv bolus complained of nausea, and 18 of these 25 experienced vomiting. Twenty-six patients had diarrhea and 14 had abdominal cramps. Seven of these patients had gastrointestinal toxic effects which were so severe that they required hospitalization or suspension of therapy or they refused further treatment. These toxic effects included dehydration secondary to diarrhea or vomiting, diffuse crampy abdominal pain, and adynamic ileus which were all reversible and dose-related. Six of 28 patients receiving ≥ 1.1 mg/m^2 (22%) experienced dose-limiting gastrointestinal toxic effects while only one of 29 patients receiving ≤ 1.0 mg/m^2 had severe toxic effects. In contrast, mild to moderate gastrointestinal toxicity occurred with approximately equal frequency at all dose levels and did not require symptomatic therapy.

Four patients reported a dry sore mouth after receiving multiple doses of > 0.9 mg/m^2, but only mild erythema of the oropharynx was observed.

Of the 14 patients receiving maytansine by 24-hour infusion, seven complained of nausea, five had vomiting, five had diarrhea, three had abdominal cramps, and two had stomatitis. Only one of these patients had gastrointestinal toxicity (diarrhea and cramps) severe enough to interfere with treatment after a dose of 1.1 $mg/m^2 \times 2$.

Neurologic Toxicity

Neurologic toxicity consisted of a peripheral, predominantly sensory polyneuropathy and an insidious generalized psychomotor depression of presumed central nervous system origin.

Among patients receiving maytansine by weekly iv bolus injection, 15 complained of paresthesias and nine had loss or diminution of the deep tendon reflexes. Polyneuropathy varied from numbness at the tips of the fingers and toes to severe burning paresthesias of the hands and feet and diffuse myalgia aggravated by any motion or touch, causing some of the patients to be bedridden and afraid to move. In contrast to neurologic toxicity of vincristine (15), loss or diminution of the deep tendon reflexes occurred in only a few of the patients with polyneuropathy, and often after the neuropathy had fully developed. Psychomotor depression with generalized weakness was reported by 26 of the 55 evaluable patients receiving maytansine by weekly bolus injections. It be-

gan insidiously and was associated with a depression of mood and affect. Typically, the patients reported difficulty in getting out of bed or getting up from a chair. Objective evidence of proximal muscle weakness was found by neurologic examination in only nine of these patients and none had evidence of distal muscle weakness.

Neurologic toxicity was primarily a function of cumulative dose received. Nine of the ten patients receiving total doses of > 6 mg/m^2 experienced neurologic toxic effects. Six of these nine patients had neurologic toxic effects which were severe enough to cause the treatment to be discontinued or delayed, whereas only six of 47 patients receiving cumulative doses of ≤ 6 mg/m^2 experienced severe neurotoxic effects (chi-square = 11.1, $P < 0.001$) (fig 1).

Neurologic toxicity was also a function of the weekly bolus dose level. Nine of the 12 patients experiencing severe neurologic toxic effects were treated at dose levels of ≥ 1.1 mg/m^2/week. This group included all of the six patients who experienced severe neurologic toxic effects at cumulative doses of < 6 mg/m^2 (fig 1). There was no correlation between neurologic toxicity and prior vinca alkaloid therapy in 31 patients.

The neurologic toxic effects in patients receiving maytansine by 24-hour infusion were similar. Generalized weakness occurred in four patients, loss or diminution of deep tendon reflexes occurred in two, and paresthesias occurred in one. The most severe toxic effects occurred in the one patient who received a cumulative dose of > 6 mg/m^2.

Results of electrophysiologic studies in ten patients are summarized in table 3. Motor nerve conduction velocities were prolonged in four patients but were abnormal in two of them prior to study. Repeat electromyograms were performed in eight patients and became abnormal or worse in four of them, showing denervation activity with fibrillations, positive sharp waves, and increased irritability. No correlation was apparent between these findings and prior vinca alkaloid therapy or clinical evidence of neurologic toxicity, except in patient 4.

Hepatic Toxicity

Increases in the serum alkaline phosphatase and transaminase concentrations of $\geq 150\%$ of the upper limits of normal were found in ten of the 55 evaluable patients receiving maytansine by weekly iv bolus. None of these patients became jaundiced or had any clinical symptoms attributable to hepatic dysfunction. Their abnormal enzyme concentrations decreased or became normal despite continued treatment at the same dose level. No increased toxicity was seen in eight patients who started therapy

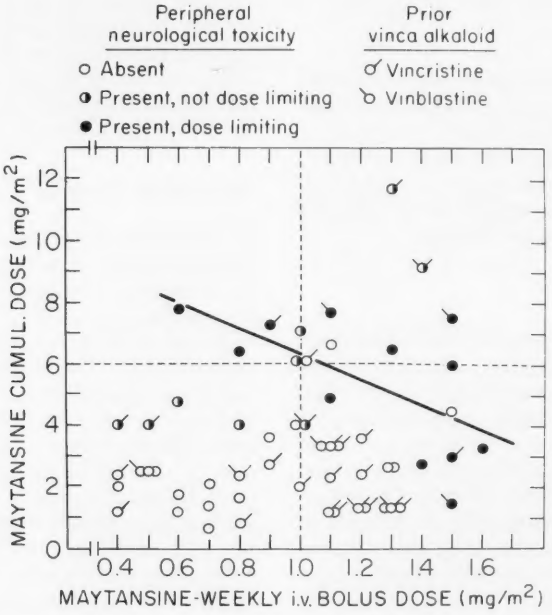

FIGURE 1.—Peripheral neurologic toxicity of weekly maytansine given by iv bolus in 57 patients. Oblique line = regression line between weekly dose (x) and cumulative dose (y) of maytansine for the 12 patients with severe neurologic toxicity (solid circles). Regression equation: $y = 10.49 - 4.15x$; $r = -0.6142$, $P < 0.05$.

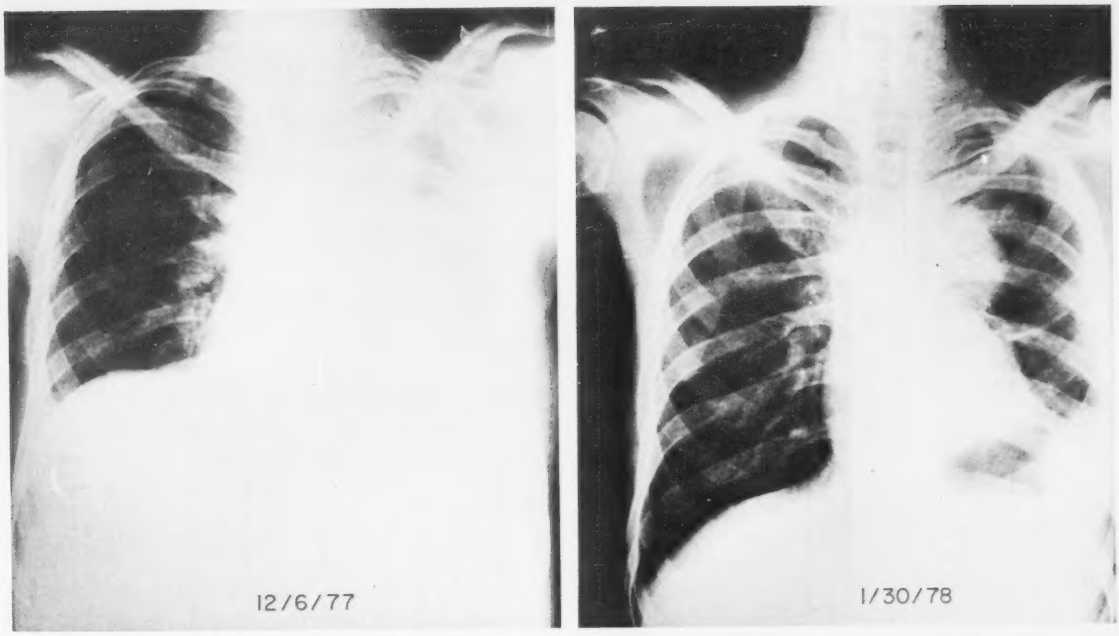

FIGURE 2.—Chest roentgenogram of a patient with invasive thymoma, epithelial type, before (left) and after (right) 5 weekly courses of maytansine at 1.1 mg/m^2 iv bolus. Marked regression of mediastinal and left pleural involvement (without thoracocentesis).

with abnormal liver function tests related to metastatic disease.

In contrast, five of the 13 evaluable patients receiving maytansine by 24-hour continuous infusion had elevated alkaline phosphatase and/or transaminase concentrations, and two of these patients became jaundiced. Both patients had had abnormal liver function tests before maytansine therapy. One of the jaundiced patients with multiple myeloma developed an enlarged tender liver, encephalopathy, and coagulation abnormalities and died on Day 12. Tests for hepatitis-associated surface antigen were negative.

Hematologic Toxicity

Only six of the 46 patients evaluable for marrow toxicity (nonleukemic patients) receiving maytansine by weekly iv bolus had leukopenia (< 4000 cells/mm^3) or thrombocytopenia (< 100,000 cells/mm^3) (table 4). One of these patients was receiving concomitant radiotherapy, and another was receiving mithramycin for hypercalcemia. Only one patient had abnormal liver function tests. In contrast, three of the six evaluable patients receiving maytansine by continuous 24-hour infusion had hematologic toxic effects, and in one of these patients particularly severe pancytopenia developed after two infusions of 1.5 mg/m^2. None of the patients experienced hepatic toxic effects.

Serial bone marrow specimens were taken over 48–96 hours from four patients with normal hematopoiesis, two with acute myelocytic leukemia, one with lymphosarcoma cell leukemia, and one with multiple myeloma in order to determine the fraction of cells in DNA synthesis (labeling index [LI]) and the mitotic index (MI). Only minor changes were induced in the LI of normal, leukemic, or myelomatous cells after a bolus injection of maytansine except for a significant decrease in the erythropoietic precursors in one patient at 24 and 48 hours. Among four patients with normal bone marrow, increases in the MI were seen at 24 hours in granulocyte precursors in two patients (MI = 2%) and in erythrocyte precursors in all four (MI ranging from 5% to 20%). These changes tended to be reversible within 48 hours. Multiple morphologic abnormalities were induced in erythrocytic nuclei at 24 and 48 hours, with abnormal mitosis, double or triple nuclei, and micronuclei. The MI of leukemic blast cells also increased from 1% before to 5% 24 hours after maytansine in the marrow of a patient with acute myelocytic leukemia. Thus, the most profound effect observed was in normal erythropoiesis, although normal granulopoiesis and leukemic myeloblasts also demonstrated stathmokinetic effects. There was no correlation with response to therapy or hematologic toxicity.

TABLE 3.—Electrophysiologic studies in 10 patients treated with maytansine

Patient No.	Sex/ age	Primary tumor	Prior vinca alkaloid (interval since last dose)	Interval between repeat electro-diagnosis (days)	Dose of maytansine × No. of wks between tests (route)	Motor nerve conduction studies	Electromyogram	Clinical findings			
								Weakness (subjective)	Weakness (objective)	Paresthesias	Decreased deep tendon reflexes
1	F/40	Breast	Vincristine (15 mos) Vinblastine (15 mos)	39	0.5 mg/m² × 5 (iv bolus)	No change	Not done	+	0	0	+
2	F/56	Thymus	None	35	1.0 mg/m² × 4 (iv bolus)	No change	No change	+	0	+	+
3	M/61	Lung	None	10	1.3 mg/m² × 1 (iv bolus)	No change, abnormal prior to study	Not done	+	0	0	0
4	F/32	Leiomyosarcoma	Vincristine (21 mos)	45	1.3 mg/m² × 6 (iv bolus)	Slowing, but remained within normal limits	Became abnormal	+	0	+	+
5	M/84	Kidney	None	15	1.4 mg/m² × 1 (iv bolus)	Slowing, abnormal prior to study	Abnormal before, worsening during study	+	+	0	+
6	F/50	Breast	Vincristine (24 mos) Vinblastine (1 mo)	30	1.4 mg/m² × 4 (iv bolus)	Slowing	No change	0	0	0	0
7	F/38	Breast	Vinblastine (1 mo)	28	1.5 mg/m² × 1 (iv bolus)	No change	No change	+	0	+	+
8	F/69	Breast	Vinblastine (2 mos)	33	1.5 mg/m² × 1 (24-hr infusion)	Slowing	Abnormal before, worsening during study	0	0	0	0
9	F/40	Breast	Vinblastine (2 mos)	22	1.5 mg/m² × 3 (iv bolus)	No change	No change	+	+	+	+
10	M/77	Colon	None	30	1.5 mg/m² × 4 (iv bolus)	No change, abnormal prior to study	Abnormal before, worsening during study	+	0	+	+

TABLE 4.—Hematologic toxicity of weekly maytansine in nonleukemic patients

Route of administration	Dose schedule (mg/m^2)	Nadir hemoglobin (g/100 ml)	Nadir wbc count* ($cells/mm^3$)	Nadir platelet count* ($cells/mm^3$)	Duration of toxicity
Iv bolus	0.6 × 8 wks	13.8	3200	387,000	†
	1.2 × 3 wks	8.0	3600	67,000‡	1 wk
	1.3 × 1 wk	8.9	3600	75,000§	3 days
	1.3 × 2 wks	11.4	2200	173,000	†
	1.5 × 3 wks	8.8	3300	630,000	1 wk
	1.6 × 1 wk	9.4	1500	280,000	†
24-hr infusion	0.5 × 6 wks	8.8	2600	101,000	2 wks
	1.3 × 1 wk	8.3	2700	52,000	4 wks
	1.5 × 2 wks	5.9	275	93,000	†

*Leukopenia is defined as < 4000 $cells/mm^3$ and thrombocytopenia is defined as < 100,000 $cells/mm^3$.
†Died within 1 wk.
‡Also received mithramycin for hypercalcemia.
§2 wks after radiotherapy to the spine.

Miscellaneous Toxicity

Thrombophlebitis of the veins or cellulitis at the site of paravenous infiltration developed in four patients treated by weekly iv bolus and in six of the 13 patients treated by 24-hour infusion. One patient developed generalized exfoliative dermatitis after seven weekly bolus injections at 0.4 mg/m^2 and had to be withdrawn from the study. Two patients developed unexplained fevers after each of two treatments, one by bolus and the other by infusion. Seven patients developed unexplained hypokalemia. Most patients who were entered in the study had alopecia from previous chemotherapy and this side effect could not be evaluated.

Therapeutic Effect

Sixty-five of the 71 patients treated were evaluable for therapeutic effect. Objective response by standard criteria (partial response > 50%) was seen in three patients including one each with thymoma (1.1 mg × 5, iv bolus) (fig 2), large cell anaplastic lung carcinoma (0.6 mg × 13, iv bolus), and diffuse histiocytic lymphoma (1.1 mg × 2, 24-hour infusion). Minor response was seen in one patient with carcinoma of the colon and in one with lymphosarcoma cell leukemia. All responses were of short duration (2–10 weeks) and occurred in three patients who previously had received vincristine. Among patients with acute myelocytic leukemia, two had normalization of peripheral hemograms, but none showed any objective improvement in the bone marrow.

DISCUSSION

The most prominent finding in the present trial was the high incidence of severe neurologic toxicity when the cumulative dose was > 6.0 mg/m^2. This relationship was present for weekly administration either by rapid iv bolus or by 24-hour continuous infusion. The 6-mg/m^2 limit for cumulative neurologic toxicity was independent of dose level for patients treated with 0.5–1.3 mg/m^2/week. Prior vincristine or vinblastine therapy, liver dysfunction, or poor performance status did not seem to predispose patients to severe or frequent toxicity. There was an increased incidence of mild to moderate neurologic toxicity in patients ≥ 65 years old.

Both the peripheral and central forms of neurologic toxicity worsened for several weeks after the drug was discontinued in severely affected patients, and resolved slowly, usually over a period of about 8 weeks. Delaying the last one or two treatments in patients reaching a high cumulative dose did not prevent the toxicity from occurring when the 6-mg/m^2 limit was eventually exceeded.

In other studies, little or no neurologic toxicity was reported, but in these studies too few patients were treated with cumulative doses of 6 mg/m^2 (6,7,9).

In one reported study of maytansine administered in 5-day courses at 3-week intervals, both central and peripheral neurologic toxic effects were found at dose levels of 0.5 mg/m^2/day × 5 days (2.5 mg/m^2/course) (8). One patient with severe polyneuropathy had marked slowing of the nerve conduction velocities (8). In contrast, in the present study, the only patient to develop significant slowing of the nerve conduction velocities had no clinical evidence of peripheral neuropathy and no diminution of the deep tendon reflexes. The present study confirms the neurologic toxicity of maytansine, which was expected from its similarities with vincristine and from data available from toxicologic animal studies (11,16). Electrophysiologic studies in ten patients did not appear to correlate with clinical neurologic toxicity. Although the number of patients is small, and the interval between tests may

have been too short to detect significant changes in most instances, such a lack of correlation was also found for vincristine (15). The preservation of conduction velocities in the presence of clinical neuropathy suggests a mechanism of axonal degeneration rather than segmental demyelination, as for vincristine (17). Indeed, the vagus nerve of cats exposed in vitro to maytansine showed a reduction in the number of microtubules and a partial blockage of fast axoplasmic transport (18).

Gastrointestinal toxicity occurred frequently in reported phase I trials of maytansine at doses of 1.6 mg/m^2 (9), 0.6 mg/m^2/day × 3 days (6,7), and 0.5 mg/m^2/day × 5 days (8). In the present study, nausea, vomiting, diarrhea, constipation, and diffuse abdominal cramps occurred with a disturbing frequency and severity at dose levels of ≥ 1.1 mg/m^2/week, requiring the hospitalization of four patients. It is possible that autonomic neuropathy accounts for some of the gastrointestinal toxicity of maytansine.

From the standpoint of gastrointestinal toxicity, the weekly administration of maytansine by bolus injection at doses ≤ 1.0 mg/m^2 is safe and can be administered on an outpatient basis.

There was no appreciable difference between the iv bolus and 24-hour infusion schedules for neurologic and gastrointestinal toxic effects. In contrast, hepatic toxic effects and myelosuppression, as well as local tissue injury by paravenous infiltration, were more common and more severe in the 24-hour infusion group. Since the therapeutic effect did not appear higher with the 24-hour infusion schedule, the iv bolus route seems preferable.

Thus, the recommended weekly schedule of maytansine for phase II trials is 1.0 mg/m^2 by iv bolus for six doses. This schedule combines the maximum cumulative dose for avoidance of severe neurologic toxicity (6 mg/m^2) with the maximum safe dose level for gastrointestinal toxicity (1.0 mg/m^2). A lower dose level would permit longer treatment. In previous studies, maytansine has been administered at higher single doses with longer intervals. The safety of these doses for gastrointestinal and cumulative neurologic toxicity is uncertain since only a few patients were treated repeatedly. A weekly schedule of maytansine at a lower dose may be safer from the standpoint of gastrointestinal toxicity.

The therapeutic effect of maytansine in these studies and early phase II studies (19–21) using 3-week schedules has been disappointing. Further investigation seems warranted, especially since the bone marrow toxicity of maytansine is minimal and allows its use in combination with other drugs for induction regimens. Moreover, there is no obvious cross-resistance between vincristine and maytansine in man, in contrast to experimental studies (9). The best response occurred in one of our patients with invasive thymoma who was previously treated with a number of chemotherapeutic agents. Since maytansine produced atrophy of the thymus in rats (11), its effect in T-cell neoplasms also deserves further study.

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Phase II Study of 4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (AMSA) in Metastatic Breast Cancer[1,2]

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SUMMARY

A phase II study of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (AMSA) was carried out in previously treated patients with metastatic breast cancer. Four of 26 evaluable patients achieved partial responses and four showed minor responses. The maximum tolerated dose of AMSA was 30 mg/m²/day × 3 days and the dose-limiting toxic effect was myelosuppression. Two of the AMSA responders had not responded to initial therapy with an Adriamycin-containing regimen, indicating a lack of cross-resistance between these two DNA binders.

[Cancer Treat Rep 63:1961–1964, 1979]

4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (AMSA), a new acridine derivative, has recently entered clinical trials. It is an inhibitor of DNA synthesis and binds to DNA by intercalation. In phase I studies, the dose-limiting toxic effect of AMSA was myelosuppression, without any other significant toxicity (1,2). Antitumor activity was reported against adenocarcinomas of the lung and ovary and lymphomas. Because of its generally mild, predictable, and reversible toxic effects, phase II studies have been initiated in a variety of solid tumors. In this paper, we report the results of a phase II evaluation of AMSA in metastatic breast cancer.

PATIENTS AND METHODS

Thirty-four patients with metastatic breast cancer were treated with AMSA. All patients had been treated previously with 5-fluorouracil, Adriamycin, and cyclophosphamide (FAC). All except three had also received methotrexate. The general characteristics of the patients are summarized in table 1. All patients had measurable or evaluable metastatic lesions and had a performance score of > 4 (Eastern Cooperative Oncology Group cri-

TABLE 1.—Patient characteristics

No. of patients entered	34
Median age in yrs (range)	51 (32–80)
No. of patients with—	
Performance score	
≤ 2	28 (82%)
3	6 (18%)
No. of metastatic sites	
1 or 2	20 (60%)
≥ 3	14 (40%)
Sites of metastases (1 or more)	
Soft tissue	25
Visceral	24
Bone	19
Prior endocrine treatment	32
Prior chemotherapy*	
F + A + C	33
F + A + C + M	31
F + A + C + M + phase II drugs†	21

*F = 5-fluorouracil, A = Adriamycin, C = cyclophosphamide, M = methotrexate.

†Phase II drugs used included 1 or more of the following: peptichemio, hexamethylmelamine, anguidine, maytansine, or hycanthone.

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teria). All patients had normal wbc count, normal platelet count, and no gross abnormalities of hepatic or renal function (serum bilirubin and serum creatinine both < 2 mg/dl).

AMSA was administered at a starting dose of 40 mg/m^2/day × 3 days and repeated at 21-day intervals. After the first few patients experienced excessive myelosuppression at this dose level, the starting dose was decreased to 30 mg/m^2/day × 3 days. The dose of AMSA was increased or decreased by 20% in order to achieve adequate myelosuppression, which was defined as the lowest neutrophil count of approximately 1000/mm^3, without causing the platelet count to decrease to < 75,000/mm^3. All patients had pretreatment staging evaluation to determine the extent of metastatic disease, and all had determinations of baseline blood counts and liver function studies. Appropriate radiologic and radioisotopic studies were repeated after two to three courses of AMSA to determine the response. Blood counts were checked before each course of treatment and once weekly between courses to determine the extent of myelosuppression and to determine the time of recovery from myelosuppression.

All measurable lesions were recorded before starting AMSA therapy and before each course of treatment. The criteria of response were standard. A partial response required a > 50% reduction in the size of all measurable lesions. A minor response was defined as objective tumor regression of > 25% but < 50%. When the lesions decreased by < 25% or there was subjective improvement accompanied by no change in tumor size for > 8 weeks, the response was recorded as stable disease. An increase in tumor size by > 25% or the development of new lesions was recorded as progression of disease. Patients who received fewer than two courses of AMSA for reasons other than obvious progression of disease were not considered evaluable for response. Patients who died of progressive disease in the first 2 weeks of the AMSA trial were labeled as early deaths and were not evaluable for response. The duration of response was measured from the time of initiation of chemotherapy to the time of progression of disease.

RESULTS

Of the 34 patients treated with AMSA, 26 were evaluable for response and 30 for toxicity. Four patients were lost to followup and were not available for evaluation after starting AMSA therapy. Three patients had an inadequate trial of AMSA because they refused treatment after one course or were changed to another form of therapy. One patient was excluded because of early death (Day 14) secondary to pulmonary embolism.

Response

Four patients (15%) had a partial response to AMSA therapy. One of these had > 90% clearing of her extensive chest wall tumor that lasted for 3 months. A second patient had complete resolution of diffuse ulcerative disease involving the left chest wall, accompanied by reduction in a pleural effusion, which lasted for 5 months. Two partial responders are still receiving AMSA therapy and continue in remission for 9 and 11 months. One of these had widespread osseous metastases and bilateral pulmonary metastases and both sites have responded. Her tumor had failed to respond to prior FAC chemotherapy. The remaining patient with extensive bone metastases has had marked relief of bone pain, accompanied by improvement in bone scan and reduction in carcinoembryonic antigen level. This patient had rapidly progressive disease that was unresponsive to three courses of FAC therapy before she was treated with AMSA.

Four patients had minor responses; all of these responses occurred in chest wall and nodal disease, whereas disease in the bone and lung in two of the patients remained stable during this period of time. The responses lasted 2–4 months. Two patients had stable disease for 5 and 8 months. All eight patients who showed evidence of response to AMSA had received prior chemotherapy with standard drugs which are part of our FAC-CMF[4] regimen (table 1).

The median survival of the 16 nonresponders was 5 months, with a range of 1 to 13+ months. The median survival of the ten responders was in excess of 8 months; only three patients have died of progressive disease at 5, 6, and 7 months.

Toxicity

The toxic effects of AMSA in 30 evaluable patients are shown in table 2. The dose-limiting toxic effect was myelosuppression which was severe at the initial dose level of 40 mg/m^2/ day × 3 days. Among the nine patients who received AMSA at this dose level the median lowest granulocyte count was 400/mm^3; three patients had no circulating granulocytes and two of these patients developed septicemia. This led to reduction of the AMSA dose to 30 mg/m^2/day × 3 days, which was also quite myelosuppressive, resulting in a neutrophil count of < 500/mm^3 in one third of the patients treated at this dose level. Only five patients tolerated repeated courses of AMSA at 40 mg/m^2/day × 3 days without excessive myelosuppression. Myelosuppression was more marked in patients who had received previous irradiation to bone marrow-containing regions of the skeleton, or had received prior treatment with drugs, such as mitomycin C or peptichemio, that cause long-lasting injury to the bone marrow. Throm-

[4]CMF = cyclophosphamide, methotrexate, and 5-fluorouracil.

TABLE 2.—Toxicity of AMSA in 30 patients

	Dose level (daily × 3 days)	
	30 mg/m²	40 mg/m²
Myelosuppressive toxicity		
No. of patients	20	9
Median lowest wbc count (cells × 10^3/mm³)	1.9	1.2
Range	0.6–3.5	0.6–3.3
Median lowest neutrophil count (cells × 10^3/mm³)	0.7	0.4
Range	0–2.2	0–1.1
Median lowest platelet count (cells × 10^3/mm³)	90	75
Range	8–250	2–220
Nonmyelosuppressive toxicity		
Nausea, vomiting	8/30 (27%)	
Dizziness, headache	5/30 (seizures in 1 patient)	

bocytopenia occurred less frequently than neutropenia but was still more marked in breast cancer patients than the experience in patients with other solid tumors treated with AMSA. The nadir of myelosuppression was generally 12–14 days after AMSA therapy, with recovery by Day 21–23. One fourth of the patients had not recovered from myelosuppression by Day 21, and accordingly received AMSA at 28-day intervals. Eleven patients received more than three courses of AMSA without showing any evidence of cumulative myelosuppression.

Other than myelosuppression, AMSA was well-tolerated. One fourth of the patients had mild nausea or occasional vomiting. Headache and dizziness were experienced by five patients. This generally occurred soon after termination of AMSA infusion and resolved spontaneously after 1–2 hours. There was no change in blood pressure to account for the dizziness. One patient experienced grand mal seizures on Day 3 of the second course of AMSA. She had felt "lightheaded" soon after AMSA infusions and seizures occurred 24 hours after the second dose of the second course. The patient was confused and incoherent for a few hours preceding the seizures. Other than a chronic low serum calcium level (8–8.5 mg/dl), no other cause for the seizure was found.

DISCUSSION

In this phase II study, AMSA has shown low but definite evidence of antitumor activity in heavily previously treated patients with metastatic breast cancer. The most remarkable feature of AMSA is its excellent tolerance by patients, which is due to a lack of significant gastrointestinal toxicity. In this regard, AMSA is quite distinctive in having severe myelosuppressive toxicity without significant gastrointestinal toxicity. The maximum tolerated dose of AMSA in this study was 30 mg/m²/day × 3 days, which is lower than the recommended dose of 40 mg/m²/day × 3 days from our phase I study. This reduction in tolerance may be due to the generally compromised bone marrow function of patients with metastatic breast cancer which is due in part to the high incidence of bone marrow invasion with this tumor, and in part to the frequent use of irradiation to palliate many patients with symptomatic bone metastases. Furthermore, the prior chemotherapy received by most patients probably reduced their bone marrow reserve. Based on these observations, it is suggested that the starting dose of AMSA for previously treated patients with breast cancer be 30 mg/m²/day × 3 days, or 90 mg/m² on the single-dose schedule.

Since there are very few chemotherapeutic agents that possess antitumor activity of this magnitude in previously treated patients with breast cancer, we are cautiously optimistic about the role of AMSA in the treatment of this disease. One of the most significant findings in this study was the achievement of objective responses with AMSA in two patients who had not responded to initial chemotherapy with the FAC regimen. This suggests a lack of clinical cross-resistance between Adriamycin and AMSA, a finding contradictory to previously reported data in the P388 mouse leukemia (3). Based on the antitumor activity of AMSA in this study, we are planning to study this drug in combination with other drugs in the treatment of breast cancer.

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Phase I Study of Hycanthone[1,2]

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SUMMARY

Hycanthone, an antischistosomal drug possessing activity against experimental murine tumors, was administered by 20-minute infusion daily × 5 days to 12 patients with advanced cancer to determine dose-limiting toxicity and to look for therapeutic effect. No objective responses were seen. The dose-limiting toxic effect was acute hepatitis occurring in two of four patients treated at 100 mg/m²/day × 5. Moderate nausea and vomiting occurring on treatment days was the only other toxic effect. Hycanthone was not myelosuppressive. Assay of plasma concentrations of hycanthone revealed a plasma half-life of 3–5 hours and no accumulation of drug over the 5 days of treatment.

[Cancer Treat Rep 63:1965–1969, 1979]

Hycanthone is the drug of choice for the treatment of infestations with *Schistosoma haematobium* and *S. mansoni* (1). It is the biologically active hydroxymethyl metabolite of lucanthone (Miracil D) (1). Hycanthone was initially evaluated as a potential antitumor drug because of structural features similar to those of other anticancer agents (a planar aromatic nucleus containing a keto group and a diethyl amino moiety in the side chain) and because, at doses used for the treatment of schistosomiasis, hycanthone does not cause hematologic toxicity (2). Hycanthone has activity against L1210 and P388 murine leukemias, particularly when given ip daily × 9 days (3).[4] Antitumor activity of hycanthone is believed to be related to its ability to intercalate DNA (4). The drug is known to cause frame shift mutations in bacteria (5) and to have mutagenic properties in mammalian cells (6). There is evidence that suggests that hycanthone is teratogenic in mice (7).

Hycanthone may enhance the biologic effects of ionizing radiation (8–10). The parent compound, lucanthone, potentiates radiation damage in in vivo and in vitro systems (8), sublethal doses of lucanthone and radiation produce substantial mortality in mice (9), and hycanthone itself has been reported to cause radiosensitization in mouse mammary tumors but not in adjacent normal tissue (10).

When used for the treatment of schistosomiasis in man, hycanthone administered as a single im dose of 3 mg/kg (maximum total dose, 200 mg) infrequently causes nausea and vomiting and anorexia and rarely leads to hepatotoxicity. The latter usually consists of asymptomatic increases in the concentrations of transaminases, although acute severe toxic hepatitis has been reported (11–13). Administration of hycanthone in large doses to dogs and monkeys produces neurologic symptoms consisting of convulsions, ataxia, tremors, muscle weakness, mydriasis, salivation and temperature fluctuations, and hepatic toxicity, expressed as increased concentrations of transaminases and alkaline phosphatase, generally without accompanying specific histologic changes in the liver.[4] There was no consistent evidence of hematologic toxicity, even at lethal doses, in the beagle or the monkey.[4]

Pharmacologic studies have been carried out in several animal species using randomly labeled tritiated hycanthone. Some studies of metabolites in bile of animals given large doses of drug have been done using thin-layer chromatography (14,15). The major route of excretion in rats, dogs, cats, rabbits, and monkeys is in bile, the majority of drug being conjugated (14,15). Fifteen metabolites have been detected in bile (15). The half-life for biliary excretion of radiolabeled material ranged from 1.6

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to 3.0 hours (15). A plasma half-life of radiolabeled material of approximately 45 minutes has been reported in the monkey (14).

We report here the results of a phase I study of hycanthone given by 20-minute iv infusion daily × 5 consecutive days and, to our knowledge, the first measurements of hycanthone concentrations in human plasma.

MATERIALS AND METHODS

All patients had histologically proven unresectable carcinoma which was refractory to standard methods of therapy or for which there was no known effective therapy. No patient had had major surgical procedures, chemotherapy, or radiation therapy within the 4 weeks before the study. Patients with severe malnutrition, persistent vomiting, gastrointestinal bleeding, a serum creatinine of > 1.5 mg/dl, a total wbc count of $< 4000/mm^3$, a platelet count of $< 130,000/mm^3$, or any elevation of direct-reacting serum bilirubin or SGOT were excluded. Patients with a past history of hepatitis or with any chronic liver disease were also excluded. All patients were ambulatory and received therapy as outpatients. Nine of the 12 patients treated with hycanthone had received previous chemotherapy. Four patients had adenocarcinoma of the colon, three had adenocarcinoma of the pancreas, two had soft tissue sarcoma, and one each had adenocarcinoma of the sublingual gland, adenocarcinoma of the stomach, and squamous cell carcinoma of the lung.

The following tests were obtained within 2 days before initiation of therapy: wbc, differential, and platelet counts, hemoglobin, urinalysis, iothalamate renal clearance, serum creatinine, SGOT, alkaline phosphatase, direct and total bilirubin, serum calcium, serum amylase, prothrombin time, glucose tolerance test, electrocardiogram, and chest roentgenogram. All tests (except chest roentgenogram and electrocardiogram), as well as a complete physical examination and interval medical history, were obtained at 3 and 6 weeks following each course of treatment. Wbc and platelet counts were obtained twice weekly and serum creatinine and SGOT were obtained once weekly throughout the study. During the 5 days of treatment, wbc and platelet counts, serum creatinine, and SGOT were obtained on the third and fifth day of treatment. When it became apparent that hycanthone could cause acute hepatotoxicity, any patient having an increase in SGOT on the third day of treatment had daily SGOT determinations prior to each subsequent day of therapy. Treatment was omitted if the SGOT was ≥ 100 units/liter (five times our upper limit of normal).

If at 6 weeks patients had no evidence of objective progression, no symptoms of deterioration, no decrease in performance status, and no significant decrease in body weight ($> 10\%$), they continued to receive treatment. Patients with no toxic effects were re-treated at the next dose level under study. Patients with definite but clinically tolerable toxic effects and improvement or stability of disease were re-treated at the same dose level. Patients experiencing severe toxic effects were not re-treated.

Hycanthone (Etrenol, Winthrop Laboratories, New York, NY) was dissolved in sterile water for injection to a concentration of 100 mg/ml and was subsequently diluted in 5% dextrose in water to a final concentration of 1 mg/ml. The appropriate dose was infused over 20 minutes. Twelve patients were treated at planned dose increments ranging from 30 to 100 mg/m^2 daily × 5 consecutive days. The starting dose was 30% of the highest nontoxic dose in the beagle dog and 60% of the highest nontoxic dose in the monkey.

Plasma concentrations of hycanthone were measured by a high-pressure liquid chromatographic assay in three patients receiving doses of 60 or 100 mg/m^2 × 5 consecutive days. Four milliliters of venous blood was withdrawn into heparinized tubes immediately prior to and at intervals over a 6-hour period following rapid iv administration of hycanthone on the first and fifth days of treatment. Single samples were obtained prior to administration of the drug on each of the other treatment days. Blood was chilled and centrifuged, and the plasma was removed. One volume of plasma was diluted with 1 volume of 0.01 N NaOH, and 2 volumes of butanol were added. The mixture was vortexed vigorously for 15 seconds. The emulsion was frozen in dry ice, thawed, and centrifuged in the cold at 2000 *g*, and the butanol layer was removed and used for assay without concentration. Efficiency of extraction ranged from 60% to 80%. Five to 20 μl of the butanol extract was injected into a Whatman Partisil PAC column and eluted with a mobile phase consisting of chloroform:methanol:29% ammonium hydroxide (92:8:0.1). Hycanthone was detected by recording its absorbance at 280 nm. Standard curves of known concentrations of hycanthone in control plasma samples based on peak height were used for calculation of drug concentrations. The assay was linear from 0.2 to 20 μg/ml of plasma. Column performance was variable from day to day necessitating that standard curves be obtained on the same day as analysis of unknown samples. Neither lucanthone nor hycanthone *N*-oxide could be used as internal standards because of unsuitable chromatographic characteristics.

RESULTS

Toxicity

Toxic effects associated with hycanthone are shown in table 1. The dose-limiting toxic effect was acute hepatic injury. Some increases in SGOT were seen at all doses

TABLE 1.—Toxicity of hycanthone

Dose (mg/m²/day × 5)	Course No.	Vomiting*	SGOT level (units/liter)†		
			Initial	Maximum (Day)	Recovery (Day)
30	1	0	16	No change	
		2+	21	31 (21)	Unknown
		0	11	17 (17)	10 (25)
60	1	1+	15	41 (4)	16 (12)
		2+	20	69 (5)	26 (10)
		0	14	No change	
		1+	13	20 (3)	13 (7)
		0	11	No change	
		0	13	100 (4)‡	46 (6); 14 (21)
	2	0	16	No change	
		0	15	20 (5)	18 (7)
		0	14	No change	
	3	0	10	26 (4)	19 (8)
	4	0	15	No change	
	5	1+	11	No change	
100	1	1+	11	22 (4)	15 (9)
		1+	16	57 (4)	21 (8)
		1+	11	Severe hepatitis	
	2	0	15	Severe hepatitis	

*0 = none; 1+ = once or twice; 2+ = more than twice but not requiring parenteral fluids; 3+ = requiring parenteral fluids; and 4+ = life-threatening.
†Upper limit of normal SGOT = 20 units/liter.
‡4th and 5th doses omitted.

tested. In four of 12 courses at 60 mg/m²/day × 5 days, the SGOT increased above the upper limit of normal of 20 units/liter to maximum values of 41, 69, 100, and 26 units/liter. These changes were detectable after the second or third day of treatment and recovery was evident a few days after treatment was discontinued. Of four courses at 100 mg/m², two were followed by mild increases in SGOT to 57 and 22 units/liter and two were followed by severe hepatitis.

A 56-year-old male with carcinoma of the colon, abdominal carcinomatosis, and ascites had severe hepatitis. A pretreatment liver scan failed to reveal the presence of metastatic disease, and liver function was normal. He had failed treatment with pyrazofurin as a single agent and methyl-CCNU, 5-fluorouracil, and vincristine as combination chemotherapy. The initial course of hycanthone at 60 mg/m²/day × 5 days resulted in no toxicity. Six weeks after the first treatment, a second course of hycanthone at 100 mg/m²/day × 5 days was followed by marked abnormalities of liver function and generalized weakness without other symptoms. The SGOT rose from a pretreatment value of 15 units/liter to a maximum value of 1235 units/liter on Day 6, the alkaline phosphatase rose from 240 units/liter (upper limit of normal) to 443 units/liter on Day 16, and the bilirubin rose from < 1 mg/dl (total) to 9.3/14.7 mg/dl (direct/total) on Day 16. The prothrombin time rose from a pretreatment value of 11 seconds (control, 11 seconds) to a maximum of 15.7 seconds on Day 16. Liver function gradually improved, and by Day 29 the serum SGOT was 61 units/liter, the alkaline phosphatase was 424 units/liter, and the total bilirubin was 3.5 mg/dl. The patient died at home 42 days after the first day of treatment. Progressive metastatic carcinoma was the cause of death. An autopsy showed extensive abdominal carcinomatosis with multiple small nodules in the liver. The hepatic parenchyma showed only nonspecific changes. The other patient developing severe hepatitis was a 63-year-old male who had pancreatic carcinoma with multiple peritoneal implants and small nodules in the liver. Ascites was present but no hepatic dysfunction was noted in pretreatment studies except for an alkaline phosphatase level of 260 units/liter. He had received no chemotherapy prior to treatment with hycanthone. After a dose of 100 mg/m²/day × 5 consecutive days, he experienced progressive weakness and anorexia. By the eighth day, the SGOT rose from a pretreatment value of 11 to 1216 units/liter, the alkaline phosphatase rose from 260 to 325 units/liter, the total bilirubin rose from 0.6 to 2.8 mg/dl, and the prothrombin time rose to 19.5 seconds. Over the next 10 days the SGOT fell to 96 units/dl, the alkaline phosphatase fell to 230 units/liter, and the total bilirubin rose to 7.5 mg/dl. On the 19th day after the first day of treatment the patient died of massive pulmonary embolization documented at autopsy. The liver was grossly normal except for small scattered tumor nodules. The abdomen contained approximately 3 liters of non-

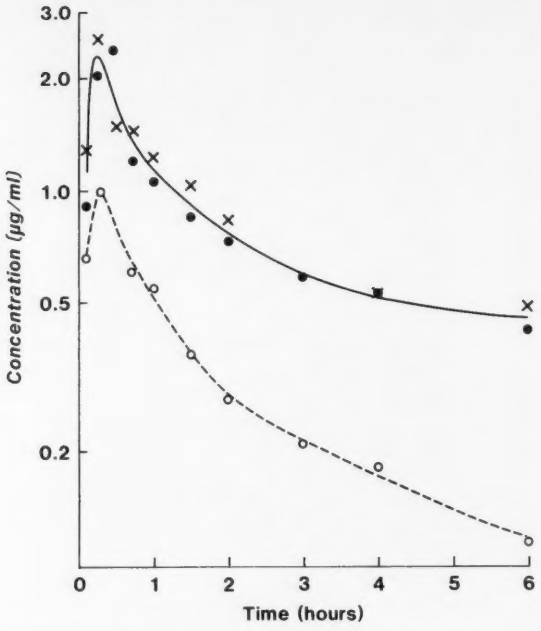

FIGURE 1.—Plasma concentrations of hycanthone following 20-min infusion at ×, ● = 100 mg/m² and ○ = 60 mg/m². Solid line = mean value for 2 patients.

bloody ascitic fluid. Histologic study of the liver showed some central lobular necrosis and foci of regenerating cells but no other evidence of acute hepatic necrosis.

The only other toxic effect was nausea and vomiting. It was generally mild, was limited to the days of treatment, and was never dose-limiting. There was no hematologic toxicity even though nine of the 12 patients had had previous chemotherapy with myelosuppressive cytotoxic drugs. Three patients received two courses of hycanthone (30 and 60 mg/m², 60 and 60 mg/m², and 60 and 100 mg/m²) and one patient received five courses (30 mg/m² and four courses of 60 mg/m²) without evidence of hematologic toxicity.

Pharmacologic Studies

Following 20-minute infusions of hycanthone on the first day of treatment, peak plasma concentrations of drug were approximately 1 μg/ml after 60 mg/m² (one patient) and 2.4 μg/ml after 100 mg/m² (two patients). As shown in figure 1, an initial rapid distribution phase was followed by one or possibly two slower phases of elimination. Assuming only one postdistributive phase of elimination, the half-life was estimated to be between 3 and 4 hours. There was no evidence of accumulation of hycanthone in plasma over 5 days of treatment in three patients treated at a dose of 100 mg/m², including one who developed toxic hepatitis.

Responses

There were no objective responses. One patient, a 70-year-old male with metastatic grade II adenocarcinoma of the colon, remained stable for 7 months while receiving five courses of hycanthone: one at 30 mg/m² and four at 60 mg/m²/day × 5 consecutive days. Three other patients progressed at 12 weeks after receiving two courses of hycanthone. The other eight patients showed progression after their first course of treatment.

DISCUSSION

Despite potential hepatic toxicity, phase II studies of hycanthone are indicated on the basis of activity against L1210 and P388 experimental leukemias, lack of hematologic toxicity, and purported radiosensitizing effects. We recommend a maximal iv dose of 60 mg/m²/day × 5 days every 3–4 weeks. This interval permits recovery of drug-induced abnormalities in liver function. An increased risk of hepatic toxic reactions in patients with pretreatment abnormalities in hepatic enzymes is possible but has not been established. Since the two patients who developed severe hepatitis had ascites, there may be an increased risk in administering hycanthone to patients with significant fluid retention.

Legha et al (16) have reported results of a phase I trial of hycanthone given daily × 5 days by infusion over 45–60 minutes rather than over 20 minutes. They studied daily doses of 30, 70, 80, and 90 mg/m² and noted dose-limiting hepatic toxic effects, frequent nausea and/or vomiting, and no hematologic toxic effects. They recommended a dose of 70 mg/m²/day × 5 days for subsequent trials (16).

Hycanthone should not be given to patients whose pretreatment SGOT or alkaline phosphatase is more than twice the upper limit of normal or who have any increase in direct serum bilirubin. All patients receiving hycanthone must have liver function monitored during treatment. We suggest that the SGOT be measured prior to the fourth dose of the drug and treatment stopped if the SGOT has increased to more than five times the upper limit of normal regardless of the pretreatment value. If a smaller increase in the SGOT is noted after the first 3 days of treatment, the SGOT should be repeated prior to administering additional drug. It seems prudent to avoid potential hepatotoxins, including phenothiazines, during the course of treatment although there are no data to support this opinion.

We are continuing our efforts to develop a more satisfactory assay for hycanthone. The current assay is suf-

ficiently sensitive to quantitate the drug at the microgram per milliliter level. However, measurement of drug is based upon the assumption that efficiency of extraction from plasma is similar from sample to sample. Although this appears to be true from our analyses of plasma samples spiked with hycanthone, a method utilizing an internal standard is required for more precise delineation of pharmacokinetic parameters.

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Treatment of Mouse Tumors With 7-Con-*O*-methylnogarol and Other Analogs of the Anthracycline Antibiotic, Nogalamycin [1,2]

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SUMMARY

The chemotherapeutic activity of analogs of the anthracycline antibiotic, nogalamycin, was investigated in the P388 and L1210 leukemias and the B16 melanoma in mice. Among the compounds tested, 7-con-*O*-methylnogarol was found to have superior activity. Depending on the route and schedule of administration, increases in lifespan (ILS) in excess of 100% were observed in all three tumor systems. Additional testing of 7-con-*O*-methylnogarol demonstrated significant activity in the murine colon 26 and colon 38 tumors and the $CD8F_1$ mammary tumor. 7-Con-*O*-methylnogarol was not significantly effective against murine Lewis lung carcinoma, although ILSs of 38% and 29% were achieved in two experiments. Activity was observed against ip inoculated P388 leukemia after ip, sc, oral, and iv drug administration. 7-Con-*O*-methylnogarol was also highly active (ILS $\geq$ 120%) after ip drug administration to mice with iv inoculated P388 leukemia. Significant ILS values resulted from a variety of schedules of administration against ip inoculated P388 leukemia and B16 melanoma. Experiments in which the time of single-dose administration was varied prior to the time of ip P388 leukemia inoculation showed that residual drug or bioactive drug-related materials remained in mice for 8 hours after 50 mg/kg administered ip and for 48 hours after 200 mg/kg administered sc.

[Cancer Treat Rep 63:1971–1978, 1979]

Nogalamycin is a fermentation-derived antitumor agent of the anthracycline type which was once considered for clinical trials but proved to be unacceptably toxic in dogs and monkeys (1). Recently, a number of analogs of nogalamycin were prepared by chemical modification, with the hope of reducing the toxicity while retaining the therapeutic activity (2,3) (fig 1). The antitumor activity of the derivatives was evaluated in three mouse tumor systems. The superior activity of one compound, 7-con-*O*-methylnogarol, stimulated further investigations of its biochemical and pharmacologic properties.[5]

In addition to its structural differences, 7-con-*O*-methylnogarol has certain biochemical properties which distinguish it from nogalamycin, Adriamycin, and other anthracycline compounds (4,5). In cell growth and macromolecular synthesis inhibition studies with L1210 cells in culture, the ID50 (drug concentration required for 50% inhibition compared to controls) for cell growth was 0.3 μM compared to > 46 μM for RNA and DNA synthesis inhibition, suggesting that cytotoxicity is not mediated through nucleic acid synthesis inhibition (4). Concentra-

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[5]7-Con-*O*-methylnogarol was previously called 7(*R*)-*O*-methylnogarol. New data have shed some doubt on the previously assigned stereochemistry of the molecule, necessitating a name change (*see* ref 3 and fig 1).

tions of 7-con-*O*-methylnogarol up to 100 μM produced 0 inhibition of highly purified DNA and RNA polymerases (5). Furthermore, 7-con-*O*-methylnogarol was found to be weakly bound to DNA (compared to Adriamycin and other nogalamycin analogs) by circular dichroism difference spectrum and DNA thermal denaturation methods and by the effect of premixing the agents and calf thymus DNA on L1210 leukemia cell growth inhibition (4). Similar studies with nogalamycin and other analogs showed that RNA synthesis and RNA polymerase activity were inhibited at cytotoxic concentrations and that these agents were strongly bound to DNA (4,5). Although recent studies have suggested multiple actions of Adriamycin (6,7), its cytotoxicity is thought to be at least partially mediated through strong binding to DNA resulting in RNA synthesis inhibition (6).

In addition to its interesting biochemical properties, 7-con-*O* methylnogarol is $< 1/15$ as potent as Adriamycin in inducing the characteristic anthracycline cardiotoxicity in rabbits (8). The antitumor activity of the nogalamycin analogs, including 7-con-*O*-methylnogarol, is described in this paper.[6]

MATERIALS AND METHODS

Agents

Nogalamycin was produced by microbial fermentation (9) and all analogs were prepared at The Upjohn Company by published methods (2,3) (fig 1). Adriamycin was provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

Structure and Nomenclature of Nogalamycin Derivatives

A scheme summarizing the preparation, structure, and names of the nogalamycin analogs is shown in figure 1. The compounds are classified into those containing a carboxyl or carbomethoxy group at C-10 (designated "-nogala-") and those in which the C-10 carboxyl or carbomethoxy group has been replaced by hydrogen (designated "-noga-"). The compounds are further divided into those containing the nogalose sugar attached through a glycosidic bond at C-7 (designated "-mycin-"), those with nogalose replaced by hydroxyl or an alkyl ether (eg, 7-con-*O*-methylnogalarol and 7-con-*O*-methylnogarol) or by hydrogen (7-deoxynogalarol and 7-deoxynogarol), and those in which the A ring has been aromatized (nogalarene and nogarene).

The stereochemical configuration of the A ring of nogalamycin and the analogs indicated in figure 1 is the most probable based on present experimental evidence. However, there is a possibility that the correct structures are the mirror images of those indicated (3).

In the course of preparing 7-*O*-alkylnogalarols and 7-*O*-alkylnogarols, it was found that two compounds isomeric at C-7 were formed (3); therefore, a further designation is necessary to indicate the relative stereochemistry at C-7 for those derivatives in which that position is a chiral center, eg, 7-con-*O*-methylnogarol and 7-dis-*O*-methylnogarol. In dis isomers the C-9 hydroxyl and the C-7 nogalose or alkoxyl substituents project from opposite sides of the A ring (fig 1). Con isomers have the 7-substituent projecting from the same side of the A ring as the C-9 hydroxyl. The parent compound, nogalamycin, has the dis configuration in the A ring. For several of the derivatives, both diastereomers have been prepared and the biologic activity of each is reported. When the chemical process did not yield enough material for testing, in vivo activity data were not obtained.

In Vitro Inhibition of L1210 Leukemia Cell Growth

The inhibition of mouse L1210 leukemia cell growth was measured in an in vitro tube dilution assay following 3 days of exposure to the test agent (10). The drug effects were expressed as ID50 and ID90 values (μM) which were obtained by plotting the logarithms of drug concentrations against percent inhibition of cell growth and interpolating the concentrations required to inhibit 50% and 90% of cell growth.

Formulations for Administration to Mice

Drugs were administered as solutions in aqueous 0.1 M glucuronic or lactic acid or as aqueous suspensions containing Tween 80. The injection volume was 0.2 or 0.5 ml/mouse.

Animals and Tumors

Standardized protocols of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, were followed for continuous passage and implantation of tumors in chemotherapy testing (11).[7] Male $B6D2F_1$ (C57BL/6 female × DBA/2 male) and $CD2F_1$ (BALB/c female × DBA/2 male) mice and female $CD8F_1$ mice were used in the experiments. Mice were 6–

[6] A preliminary account of this work was presented at the 68th annual meeting of the American Association for Cancer Research, Denver, CO, May 18–21, 1977 (Neil GL, Blowers CL, Houser DJ, et al. Antitumor activity of some new analogs of nogalamycin. Proc Am Assoc Cancer Res and ASCO 18:2, 1977).

[7] Instruction 271b, Summary of the Usual Characteristics of Selected Murine Models Used Under the Auspices of the NCI Division of Cancer Treatment. Prepared by the National Cancer Institute, Bethesda, MD, Apr 1, 1978.

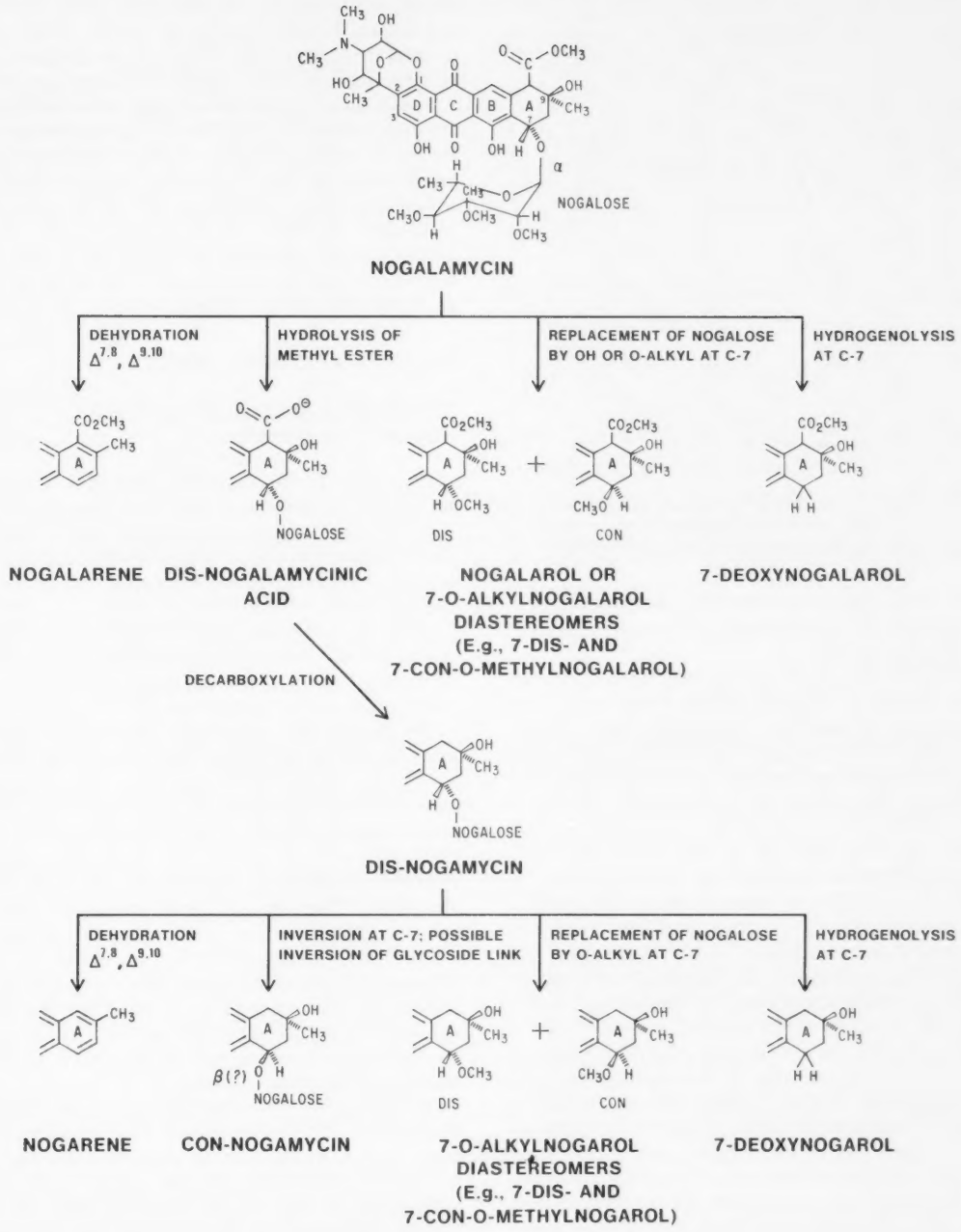

FIGURE 1.—Preparation, structure, and nomenclature of nogalamycin analogs. The stereochemical configuration of the A ring of nogalamycin and the analogs indicated is the most probable based on present experimental evidence. However, there is a possibility that the correct structures are the mirror images of those shown (*see* ref 3).

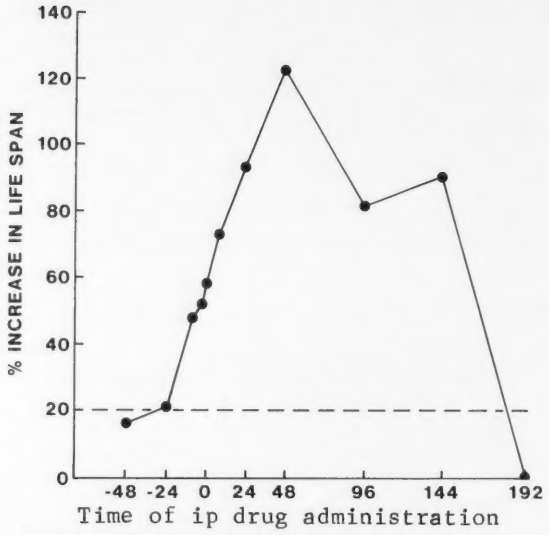

FIGURE 2.—Influence of time of ip drug administration relative to time of iv P388 leukemia inoculation on the chemotherapeutic activity of single doses of 7-con-*O*-methylnogarol.

8 weeks of age and weighed 18–25 g. Groups of six to ten mice were housed in plastic cages and were given pelleted food and water ad libitum.

P388 and L1210 leukemias were maintained by continuous ip passage in syngeneic DBA/2 female mice. P388 leukemia was implanted ip or iv in $CD2F_1$ mice (10^6 cells/mouse); final evaluation of each experiment was on Day 30. L1210 leukemia was implanted ip in $CD2F_1$ mice (10^5 cells/mouse); final evaluation of each experiment was on Day 60. Adriamycin-resistant P388 leukemia (P388/ADR) was developed as previously described (12); P388/ADR leukemia cells were implanted ip (10^6 cells/mouse) in $B6D2F_1$ mice and final evaluation was on Day 60. B16 melanoma, colon 38 tumor, and Lewis lung carcinoma were carried sc in syngeneic C57BL/6 male mice. B16 melanoma was implanted ip or sc in $B6D2F_1$ mice (0.5 ml of 1:10 or 1:20 wt/vol tumor brei) and final evaluation was on Day 60. Colon 38 tumor fragments were implanted with a 13-gauge trocar sc in $B6D2F_1$ mice (70 mg/mouse); final evaluation was on Day 20. Lewis lung carcinoma was implanted iv in $B6D2F_1$ mice (10^5 cells/mouse) as previously described (13); final evaluation was on Day 60. Colon 26 tumor was carried sc in BALB/cJ mice and was implanted ip (0.5 ml of 1:100 wt/vol tumor brei) in $CD2F_1$ mice; final evaluation was on Day 60. $CD8F_1$ mammary tumor was a first-generation transplant of spontaneous tumor in female $CD8F_1$ mice. Tumors were implanted sc (1:20 wt/vol tumor brei or 5×10^6 cells) in $CD8F_1$ mice. Final evaluation was on Day 30.

The response measured was median or mean survival time (in days) in all tumor systems with the exception of the $CD8F_1$ mammary and colon 38 tumors, in which the median tumor weight was measured on the day of final evaluation. Percent increase in lifespan (ILS) was calculated as (survival time of treated group/survival time of control group – 1) × 100%. For tumor growth inhibition models, percent T/C values were calculated as (median tumor weight of treated group/median tumor weight of control group) × 100%. The criteria for significant activity were as follows (listed as tumor, site, and minimum percent ILS or T/C): P388, ip or iv, 20% ILS; L1210, ip, 25% ILS; colon 26 tumor, ip, 30% ILS; Lewis lung carcinoma, iv, 40% ILS; B16 melanoma, ip, 25% ILS; B16 melanoma, sc, 40% ILS; colon 38 tumor, sc, ≤ 42% T/C; $CD8F_1$ mammary tumor, sc, ≤ 42% T/C.

RESULTS AND DISCUSSION

Comparative Antitumor Activity of Nogalamycin Derivatives

The in vitro L1210 leukemia growth inhibitory activity and the in vivo antitumor activity of analogs of several structural types are shown in table 1. Table 2 compares the in vitro and in vivo activities of the two C-7 diastereomers of several 7-*O*-alkylnogalarols and 7-*O*-alkylnogarols.

There was some correlation between the in vitro and in vivo activity. Analogs with high ID50 values were not very active in vivo; however, a low ID50 value (< 0.2 μM) did not necessarily indicate high in vivo activity.

The most active compounds against P388 leukemia were, in decreasing order, 7-con-*O*-methylnogarol, dis-nogamycin, 7-con-*O*-methylnogalarol, 7-dis-*O*-methylnogarol, 7-con-*O*-ethylnogarol, and 7-dis-*O*-methylnogalarol. Against the B16 melanoma the most active compounds were 7-con-*O*-methylnogarol, dis-nogamycin, 7-dis-*O*-methylnogarol, nogalamycin, and 7-dis-*O*-methylnogalarol. Thus, except for dis-nogamycin, the most active analogs did not contain the nogalose moiety.

The 7-deoxy compounds, 7-deoxynogalarol and 7-deoxynogarol, were only marginally active against P388 leukemia and the optimum doses were high (50–100 mg/kg) compared to the other analogs. Neither of the aromatized analogs (nogalarene and nogarene) showed much activity against P388 leukemia.

Dis-nogamycin was more active than con-nogamycin against both P388 leukemia and B16 melanoma.[8] However, 7-con-*O*-methylnogarol and 7-con-*O*-methylnogalarol were considerably more active than their corresponding 7-dis isomers against P388 leukemia. Against B16 melanoma, no difference was apparent between 7-con- and 7-dis-*O*-methylnogarol; 7-con-*O*-meth-

[8]The compound called con-nogamycin may differ from dis-nogamycin in the configuration of the glycoside linkage in addition to the C-7 stereochemical configuration (*see* ref 3).

TABLE 1.—In vitro and in vivo antitumor activity of nogalamycin and analogs[a]

Compound	NSC No.	L1210 leukemia in vitro[b]		P388 leukemia		L1210 leukemia		B16 melanoma[e]	
		ID50 (μM)	ID90 (μM)	OD[c]	ILS[d]	OD	ILS	OD	ILS
Nogalamycin	70845	0.078	0.18	1	49	1	30	0.5–1	72–157
Dis-nogalamycinic acid	265449	1.00	1.94	20[f]	56	20	18	Not tested	
Dis-nogalarol	86005	0.89	2.39	200	0[g,h]	400	0[h,i]	Not tested	
7-Dis-*O*-methylnogalarol	102815	0.27	1.67	25	60	50	12	50	84
7-Con-*O*-methylnogalarol	293862	0.13	0.30	25	98 (1/6)	Not tested		10	35
7-Deoxynogalarol	114345	0.58	1.51	50	27	400[j]	4	Not tested	
Nogalarene	82892	0.24	0.58	70	50	400[j,k]	2	Not tested	
Dis-nogamycin	265450	0.18	0.27	5	93	2.5	27	2–5	61–260
Con-nogamycin[l]	308326	0.56	1.37	25	44	Not tested		15	28
7-Dis-*O*-methylnogarol	293863	0.41	0.91	12.5	76	Not tested		16	105
7-Con-*O*-methylnogarol	269148	0.061	0.13	12.5	197 (3/6)	12.5	140 (1/6)	6–12	60–114 (1–2/10)
7-Dis-*O*-ethylnogarol	308313	0.43	1.42	Not tested		Not tested		12	34
7-Con-*O*-ethylnogarol	282724	0.16	0.32	6.5	63	Not tested		12	25
7-Deoxynogarol	308329	0.94	1.76	100[f]	21	Not tested		Not tested	
Nogarene	269149	0.22	0.37	12.5	17	Not tested		Not tested	

[a] Tumors were inoculated ip.

[b] ID50 and ID90 = concentrations required for 50% and 90% inhibition, respectively, of L1210 leukemia cell growth with 3 days of exposure to the agent.

[c] OD = optimal dose in mg/kg/injection, qd × 9 (1), ip, unless otherwise noted.

[d] % ILS based on median or mean survival times of treated and control groups; Nos. in parentheses = No. of cures/total No. treated.

[e] Because of greater variability in B16 melanoma testing, a range of values is given when possible.

[f] q4days × 3 (1) schedule.

[g] qd × 10 (1) schedule.

[h] Highest non-toxic dose tested.

[i] Conducted in $B6D2F_1$ mice.

[j] qd × 1 (2) schedule.

[k] Highest dose tested.

[l] Con-nogamycin may differ from dis-nogamycin in the configuration of the glycosidic linkage in addition to the C-7 stereochemical configuration.

TABLE 2.—Influence of C-7 substituent and configuration on in vitro and in vivo activity of nogalarol and nogarol analogs of nogalamycin[a]

C-7 substituent	Nogalarol series				Nogarol series			
	Dis[b]		Con[b]		Dis		Con	
	ID50[c]	ILS-OD[d]	ID50	ILS-OD	ID50	ILS-OD	ID50	ILS-OD
Hydroxy	0.89	0–200[e]	—[f]	—	—	—	—	—
Methoxy	0.27	60–25	0.13	98(1/6)–25	0.41	76–12.5	0.061	197(3/6)12.5
Ethoxy	0.16	NT[g]	0.15	70–12.5	0.43	NT	0.16	63–6.5
n-Propoxy	0.16	NT	0.30	NT	0.33	50–3.1	0.10	34–6.3
Isopropoxy	—	—	—	—	—	—	0.15	20–4

[a] NSC Nos. not listed in table 1 are: (*S*)-nogalarol, NSC-86005; 7-con-*O*-ethylnogalarol, NSC-293866; 7-con-*O*-*n*-propylnogalarol, NSC-293867; 7-dis-*O*-*n*-propylnogarol, NSC-293869; 7-con-*O*-*n*-propylnogarol, NSC-293868; 7-con-*O*-isopropylnogarol, NSC-282725. NSC Nos. have not been assigned to 7-dis-*O*-ethylnogalarol and 7-dis-*O*-*n*-propylnogalarol.

[b] Dis and con refer to the stereochemical configuration at C-7.

[c] ID50 = concentration (μM) required for 50% inhibition of L1210 leukemia cell growth in vitro with 3-day drug exposure.

[d] ILS-OD = % ILS (No. of cures/total No. treated) at the optimum dose (OD) in mice receiving ip inoculations of P388 leukemia when tested on a qd × 9 (1) schedule.

[e] qd × 10 (1) schedule.

[f] — indicates the compound has not been prepared.

[g] NT = not tested.

TABLE 3.—Summary of mouse therapy studies with 7-con-*O*-methylnogarol[a]

Tumor				Experiment 1		Experiment 2	
System	Site[b]	Drug route	Schedule[c]	OD[d] (mg/kg/injection)	ILS-OD[e]	OD (mg/kg/injection)	ILS-OD
P388	ip	ip	qd × 9 (1)	12.5	185 (3/6)	12.5	197 (2/6)
			q4days × 3 (1)	50	152	30	171 (2)
			qd × 1 (1)	200	75 (1/6)	50	97
			q4days × 3 (5)	150	84	75	71
		sc	qd × 1 (1)	200	56	200	93
		oral	qd × 9 (1)	12.5	37	12.5	48
		iv	qd × 1 (3)	25	27		
	iv	ip	qd × 9 (1)	12.5	187	12.5	120
			qd × 1 (1)	50	93	50	90
P388/ADR	ip	ip	q4days × 3 (1)	30	16		
L1210	ip	ip	qd × 9 (1)	12.5	140 (1/6)	12.5	104 (1/6)
			qd × 1 (4)	50	38		
Colon 26	ip	ip	q4days × 2 (1)	25	106 (3/10)	25	61 (2/10)
Lewis lung	iv	ip	qd × 9 (1)	12.5	38	12.5	29
B16	ip	ip	qd × 9 (1)	12.5	109 (1/10)[f]	6.25	77 (2/10)
			q4days × 3 (1)	18	58		
			qd × 1 (1)	100	59	200	77 (1)
			q4days × 3 (5)	10.8	103 (4/10)		
			qd × 1 (5)	400	35	400	25
	sc	ip	qd × 9 (1)	12.5	14	6.25	4
				OD	*T/C*[g]	*OD*	*T/C*
Colon	sc	ip	q7days × 2 (2)	25	21 (2/10)	50	1
$CD8F_1$ mammary	sc	ip	q7days × 5 (1)	25	1	50	1 (1/10)

[a] If > 2 experiments were performed, the results from those giving the best responses are shown. Numerous experiments have been performed in mice receiving ip inoculations of P388 leukemia, L1210 leukemia, and B16 melanoma and treated ip qd × 9 (1). Median and range of ILSs observed and No. of tests performed are as follows: P388, 131% (90%–197%), 17 experiments; L1210, 93% (77%–140%), 5 experiments; B16: 89% (60%–114%), 7 experiments.

[b] Site of tumor inoculation.

[c] qd × 9 (1) means that doses were administered every day for 9 days starting on the 1st day after tumor inoculation; q4days × 3 (5) means that doses were administered every 4th day for a total of 3 injections beginning on the 5th day after tumor inoculation; etc.

[d] OD = optimal dose.

[e] ILS-OD = % ILS (No. of cures/total No. treated) at the OD calculated from median (or mean for L1210) survival time.

[f] Cure occurred at 6.25 mg/kg.

[g] Ratio of median tumor weights of treated and control groups × 100%; Nos. in parentheses = No. of animals without palpable tumors on day of final evaluation/total No. treated.

ylnogalarol was less active than 7-dis-*O*-methylnogalarol. Higher homologs in the 7-*O*-alkylnogarol series (ethyl and propyl) retained the in vitro growth inhibitory activity (table 2), but the P388 leukemia and B16 melanoma activity in vivo declined.

Of the analogs tested, 7-con-*O*-methylnogarol and dis-nogamycin were considered the best compounds for further investigation. Despite strong activity against P388 leukemia and B16 melanoma, dis-nogamycin was only marginally active against L1210 leukemia and was inactive when tested further in the colon 26, colon 38, and $CD8F_1$ mammary tumors, and in the Lewis lung carcinoma (data not presented). 7-Con-*O*-methylnogarol has a rather broad spectrum of activity in mouse tumors (table 3). In addition to activity in the P388 and L1210 leukemias and in B16 melanoma, 7-con-*O*-methylnogarol was significantly active against the murine colon 26 and colon 38 tumors, as well as the $CD8F_1$ mammary tumor. It was not considered effective against Lewis lung carcinoma, although 38% and 29% ILS values were achieved in two experiments; 40% ILS is the criterion of activity. 7-Con-*O*-methylnogarol was not significantly active against P388/ADR leukemia, a subline of P388 leukemia resistant to Adriamycin and cross-resistant to a spectrum of other

TABLE 4.—Comparative antitumor activity of 7-con-*O*-methylnogarol and Adriamycin

Tumor			7-Con-*O*-methylnogarol		Adriamycin	
System	Site	Schedule	Dose[a]	ILS[b]	Dose	ILS
P388	ip	qd × 9 (1)	25	Toxic	2	7
			12.5	168 (1/6)	1	158 (1/6)
			6.25	111	0.5	137 (1/6)
			3.13	61	0.25	61
P388	ip	q4days × 3 (5)	160	–3	16	9
			80	59	8	40
			40	69	4	31
			20	33	2	31
P388	iv	qd × 9 (1)	25	Toxic	2	29
			12.5	187	1	10
			6.25	53	0.5	3
			3.13	25	0.25	8
L1210	ip	qd × 9 (1)	25	Toxic	2	40 (2/6)
			12.5	104 (1/6)	1	38 (1/6)
			6.25	55	0.5	29
			3.13	42	0.25	20
B16	ip	qd × 9 (1)	20	Toxic	2	158 (1/10)
			10	114	1	166 (2/10)
			5	88	0.5	160
			2.5	64	0.25	127

[a] mg/kg/injection, ip.
[b] % ILS calculated from median survival times; Nos. in parentheses = No. of cures/total No. of mice in group.

anthracycline analogs, DNA intercalators, mitotic spindle inhibitors, and protein synthesis inhibitors (12). In other studies in our laboratories, certain biochemical differences were detected between 7-con-*O*-methylnogarol and the other nogalamycin analogs. As noted above, these differences suggest that 7-con-*O*-methylnogarol may have a different mechanism of action from the other nogalamycin analogs and, possibly, from Adriamycin (4,5). Because of the interesting pharmacologic and biochemical properties of 7-con-*O*-methylnogarol, further experimental therapeutic studies were conducted.

Comparative Antitumor Activity of 7-Con-*O*-methylnogarol and Adriamycin in P388 and L1210 Leukemias and B16 Melanoma

Table 4 shows dose-response comparisons of 7-con-*O*-methylnogarol and Adriamycin. The two agents appeared to be about equally effective against ip inoculated P388 leukemia. However, whereas Adriamycin was only marginally active against iv inoculated P388 leukemia, 7-con-*O*-methylnogarol was highly active (187% ILS). 7-Con-*O*-methylnogarol was more effective than Adriamycin when delayed treatment was used against established disease [q4days × 3 (5) schedule].[9] 7-Con-*O*-methylnogarol appeared to be more active than Adriamycin against ip inoculated L1210 leukemia and less active against ip inoculated B16 melanoma.

Schedule Dependence of Activity in P388 Leukemia and B16 Melanoma

As noted in table 3, 7-con-*O*-methylnogarol was most active against both tumors when administered on a qd × 9 (1) schedule. Against P388 leukemia the q4days × 3 (1) schedule was also highly active (171% ILS) but no cures were observed. In the P388 leukemia system, the total optimal dose for the qd × 9 (1) regimen (112.5 mg/kg) was somewhat lower than that for the q4days × 3 (1) regimen (150 mg/kg) or the optimal single dose (200 mg/kg). In the B16 melanoma system, schedules other than qd × 9 (1) were significantly but not highly active, with the exception of delayed treatment against established disease [q4days × 3 (5) schedule] in which activity comparable to that of the qd × 9 (1) schedule was noted.

Influence of Routes of Tumor Inoculation and Drug Administration on Activity Against P388 Leukemia

7-Con-*O*-methylnogarol was significantly active against ip inoculated P388 leukemia when given by the iv, sc, and oral routes, but the activity was not as great as when the drug was administered ip.

[9] Abbreviations for schedules are explained in table 3.

As noted above, 7-con-*O*-methylnogarol was quite active (187% and 120% ILS in two experiments) when administered ip against iv inoculated P388 leukemia (table 3).

Influence of Time of Drug Administration Relative to Time of Tumor Inoculation on Activity Against P388 Leukemia

To obtain a crude estimate of the clearance of 7-con-*O*-methylnogarol by the mouse, experiments were performed to assess the activity of the agent administered at different times relative to tumor inoculation. A single 50-mg/kg dose was administered ip at various times (1, 2, 4, 8, 24, 48, and 96 hours) before ip inoculation of P388 leukemia, and the activity was compared to that observed when the agent was administered at the time of tumor inoculation, yielding a 266% ILS. The therapeutic effect declined as the time of drug administration before tumor inoculation increased (eg, 85% ILS at –2 hours; 50% ILS at –8 hours). 7-Con-*O*-methylnogarol was ineffective when injected ip ≥ 24 hours before inoculation. The antitumor effect was also reduced when the agent was given *after* tumor inoculation, but the response was still significant (28% ILS) when the agent was administered as much as 8 days *after* inoculation.

Sc injection of 200 mg/kg of 7-con-*O*-methylnogarol gave a 65% ILS when tumor and drug were injected simultaneously. The therapeutic response declined as the time of drug administration before tumor inoculation increased, but a 22% ILS resulted from injection at –96 hours. The response was somewhat higher when the agent was administered at +24 hours (93% ILS) than when the agent was administered at the same time as tumor inoculation.

Figure 2 shows the effect of a single 50-mg/kg dose given at various times relative to iv P388 inoculation. A marginal effect (21% ILS) was obtained with injection of drug 24 hours before tumor inoculation. The effect increased to about 60% ILS with simultaneous agent administration and tumor inoculation and then increased further to 122% ILS at +48 hours. A 90% ILS was obtained when the agent was given 4 days after tumor inoculation, a most significant result because at that point the tumor was far-advanced; the control median day of death was 8 days.

CONCLUSION

Chemical transformation of nogalamycin has resulted in a series of analogs with different biochemical properties (4,5) and greatly improved antitumor activity. The relationship between the structure and biologic activity of these compounds, however, is not simple. Among the analogs tested, 7-con-*O*-methylnogarol has superior activity. This agent, when tested in multiple doses in rabbits, did not cause the severe acute toxicity observed previously with nogalamycin in dogs and monkeys and was found to be < 1/15 as potent as Adriamycin in inducing the characteristic anthracycline cardiomyopathy (8). Because of the interesting biochemical, pharmacologic, and toxicologic properties of 7-con-*O*-methylnogarol, further preclinical development of the agent is underway.

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Treatment of Advanced Gastric Carcinoma With 5-Fluorouracil: A Randomized Comparison of Two Routes of Delivery [1,2]

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SUMMARY

Fifty-seven patients with advanced gastric carcinoma were randomized to receive 15 mg/kg/week of 5-fluorouracil by either the iv or oral route. Toxic effects of treatment included nausea and vomiting (40% with the oral route versus 35% with the iv route) and myelosuppression, with a wbc count of $< 4000/mm^3$ (28% with the oral route versus 32% with the iv route). The frequency of partial response was 12% (three of 25 patients) for the oral route and 16% (five of 32 patients) for the iv route. Only two of 36 patients with liver metastases responded. No advantage was seen for the oral versus the iv route of 5-fluorouracil in the treatment of advanced gastric carcinoma.

[Cancer Treat Rep 63:1979–1981, 1979]

Despite > 20 years of clinical use, the optimal dose and route of administration of 5-fluorouracil (5-FU) have not been determined for all disease types. In both colon (1–3) and pancreatic (4) carcinoma, the iv route has resulted in increased response frequency when compared to the oral route in randomized trials. In breast cancer, however, no clear superiority for the iv route has been demonstrated (1). In the present study, patients with advanced gastric carcinoma were randomized to receive 5-FU weekly by either the iv or the oral route.

MATERIALS AND METHODS

Patients with measurable gastric carcinoma not amenable to primary surgical or radiation therapy were eligible for entry in this study. No patients had received prior chemotherapy. Pretreatment requirements included a leukocyte count of at least $4000/mm^3$, a platelet count of $150{,}000/mm^3$, and a serum creatinine level of < 1.8 mg/100 ml. No minimum performance status was required. Biopsy-proven gastric carcinoma was obtained in all of the 57 patients (laparotomy, 40 patients; endoscopy, 14; peritoneoscopy, one; and upper GI plus lymph node biopsy, two). Pretreatment studies included complete medical history and physical examination, cbc, serum creatinine, bilirubin, alkaline phosphatase, SGOT, urinalysis, chest roentgenogram, and liver scan. Weekly hemograms were performed prior to the administration of chemotherapy. Other studies were repeated as indicated to document response or progression of disease.

Responses were classified as complete or partial. Partial responses consisted of the reduction in tumor size by ≥ 50% of the cross-sectional area of all measurable lesions with the appearance of no new lesions for a period of ≥ 1 month. No response consisted of minor change, no change, or progression of any measurable lesions during therapy.

Patients were randomly assigned to receive 5-FU either orally or iv. The dose for both groups was 15 mg/kg/week. Oral 5-FU was administered as the iv preparation diluted in tap water. After four doses, if no response or toxicity was observed, the dose was escalated to 20 mg/kg. Treatment was continued until disease progression occurred.

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TABLE 1.—Pretreatment characteristics and response to 5-FU of patients with advanced gastric carcinoma

Characteristic	5-FU route	
	Oral (25 patients)	Iv (32 patients)
Median age in yrs	55	60
Males (%)	68	76
Median Karnofsky performance status	75	65
Median pretreatment Hgb (g/100 ml)	11.6	11.4
No. of patients (%) with—		
Liver metastasis	14 (56)	22 (68)
Partial objective response	3 (12)	5 (16)
Median survival in mos	4.1	3.9

RESULTS

Clinical details of the 57 patients studied are presented in table 1. The group receiving iv treatment was somewhat older, had a higher incidence of liver metastasis, and had a lower median performance status. All of the patients were evaluable for toxicity and response. No complete responses were observed in either group. Partial responses were seen in three of 25 patients (12%) treated with oral 5-FU and in five of 32 patients (16%) treated with iv 5-FU (table 1). Responding patients lived significantly longer than nonresponders (5.3 versus 3.1 months; $P < 0.05$). Of the 36 patients with liver metastases, only two responded, both of whom were in the group receiving iv treatment. None of the pretreatment characteristics, including sex, age, performance status, initial Hgb, initial wbc count, liver metastasis, and routes of treatment, could be significantly correlated with survival. The incidence of toxic effects of oral and iv administration were, respectively: gastrointestinal, 40% versus 35%; myelosuppressive (wbc count $<4000/mm^3$), 28% versus 32%; and neurotoxic, 0 versus 8%.

DISCUSSION

Randomized studies comparing iv and oral 5-FU dose regimens have been reported for several gastrointestinal malignancies. In colon carcinoma (1–3), hepatocellular carcinoma (5), and pancreatic carcinoma (4), randomized trials have reported improved response frequency using the iv route of drug administration. In these trials of gastrointestinal malignancies, the demonstration of superiority for any regimen has been hindered by the low response frequencies observed. Similarly, in the present study of gastric carcinoma, iv 5-FU resulted in a somewhat greater response frequency than oral 5-FU. In particular, no patient with liver involvement responded to oral treatment. Since the two routes of 5-FU administration had similar hematologic and gastrointestinal toxicity, there appears to be no advantage in using the oral route of 5-FU in advanced gastric carcinoma.

In this study, 32 patients received weekly iv 5-FU treatment, and 16% achieved an objective tumor response. This result can be compared to other randomized studies in which patients with gastric carcinoma received iv 5-FU. Loading courses were employed by Kovach et al (6) and Reitemeier et al (7), with responses seen in nine of 39 patients (23%). Baker et al (8) used weekly 5-FU, and six of 29 patients (21%) responded. A recent Eastern Cooperative Oncology Group trial reported a median survival of 27 weeks for > 50 patients treated with a loading dose of iv 5-FU (9). In the present report, both the response (16%) and survival (3.9 months) of the treatment with weekly iv 5-FU were somewhat lower than those cited above; however, the large proportion of patients with liver metastases in this series indicates a group with an extensive tumor burden. In this report, malignant hepatomegaly was quite resistant to 5-FU therapy whereas a previous study noted a relatively high response frequency for hepatic indicator lesions in gastric carcinoma patients (10).

In summary, the results of all published studies of 5-FU in gastric cancer indicate the limited use of this drug when used as a single agent. In addition, no advantage was observed in using the oral route of 5-FU delivery for patients with this disease.

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Combination Chemotherapy With Mitomycin C, Adriamycin, and Cyclophosphamide in Advanced Adenocarcinoma and Large Cell Carcinoma of the Lung [1,2]

Brief Reports and Preliminary Communications

Roberto J. Fraile,[3,*] Michael K. Samson,[3] Laurence H. Baker,[3] and Robert W. Talley[4,5]

SUMMARY

Thirty-six patients with advanced carcinoma of the lung (30 with adenocarcinoma and six with large cell carcinoma) were treated with a combination of mitomycin C, Adriamycin, and cyclophosphamide (MAC) in a phase II study. Seven partial remissions were observed in adenocarcinomas, while none were seen in large cell carcinomas. The survival of patients in remission was clearly prolonged ($P < 0.01$), with responders living a median of at least 39 weeks compared to 17 weeks for nonresponders. The combination was well-tolerated with moderate anorexia, nausea, vomiting, and alopecia. Myelosuppression was manageable but was more pronounced in previously chemotherapeutically treated patients. MAC offers a reasonable response rate in patients with adenocarcinoma of the lung with significant prolongation of survival; however, there was no significant advantage when compared to mitomycin C used as a single agent.

[Cancer Treat Rep 63:1983–1987, 1979]

Lung cancer is the most common malignancy in American men and the third most common in women. An estimated 100,000 new cases of lung cancer were expected in 1978 (1) with approximately an equal number of deaths, since only 5%–9% (2,3) of patients will survive 5 years after diagnosis. Adenocarcinoma, a histologic type becoming progressively more prevalent in recent years, represents 10%–33% of all cases and large cell carcinoma represents 10%–22% (2–4).

Therapy for advanced carcinoma of the lung has remained relatively ineffective (5,6), with single agents inducing remissions in about 20% of the patients (5) but without any meaningful prolongation of survival. Combining these active agents, as has been done in lymphomas, breast carcinoma, small cell carcinoma of the lung, and other tumors, appeared to be one approach to this problem.

Mitomycin C, an antibiotic with alkylating activity isolated from *Streptomyces caespitosis*, is an effective agent in adenocarcinoma and large cell carcinoma of the lung, with a reported response rate of 16%–25% (5–8). In a study conducted recently in our institution, a 25% response rate was observed (9), but the duration of response was brief. In addition, cumulative myelotoxicity limited prolonged administration of the drug. In an attempt to mitigate this problem and to improve response rates, response duration, and survival, we combined mitomycin C with two other active agents, Adriamycin, a derivative of the antitumor antibiotic daunorubicin with a reported response rate of 15%–20% (5,10,11), and cyclophosphamide, the most widely used alkylating agent in lung cancer, with an overall response rate of 23% (3,5,12).

MATERIAL AND METHODS

All patients entered in the study had histologically proven incurable adenocarcinoma or large cell carcinoma of the lung (pathology slides were reviewed by Dr. Richard Pollard of the Pathology Department, Harper Division of Harper-Grace Hospitals). The patients were informed as to the investigational nature of the study and gave written consent in accordance with our institutional

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policy. Other criteria for eligibility included no previous therapy with any of the agents in the combination, an expected survival of at least 12 weeks, a performance status of ≥ 40% (Karnofsky scale), presence of measurable disease, initial wbc count of at least 4000/mm^3 and platelet count > 100,000/mm^3, serum bilirubin ≤ 2.0 mg/100 ml, serum creatinine ≤ 1.7 mg/100 ml, and no chemotherapy or radiotherapy within the previous 4 weeks and evidence of hematologic recovery from any therapy received > 4 weeks previously.

Patients were evaluated while receiving therapy with serial cbcs, chest roentgenograms, liver and renal function studies, tumor measurements, and determinations of performance status. One complete course of therapy (one dose of mitomycin C and two doses of cyclophosphamide and Adriamycin) constituted an adequate trial.

Poor-risk patients were defined as those ≥ 65 years of age and/or those with prior extensive chemotherapy or radiation therapy to bone marrow-bearing areas. Mitomycin C was given at 6-week intervals at a dose of 7 mg/m^2 (5 mg/m^2 for poor-risk patients). Adriamycin, at a dose of 40 mg/m^2 (30 mg/m^2 for poor-risk patients), and cyclophosphamide, at a dose of 400 mg/m^2 (300 mg/m^2 for poor-risk patients), were administered at 3-week intervals. Subsequent doses were adjusted according to nadir blood counts and patient tolerance.

Complete remission was defined as the disappearance of all clinical evidence of active tumor for a minimum of 4 weeks together with a disappearance of all tumor-related symptoms. Partial remission was defined as a ≥ 50% decrease in the sum of the products of the largest perpendicular diameters of all measured lesions lasting at least 4 weeks in the absence of a significant increase in any one lesion or the appearance of new lesions. Progressive disease was defined as an unequivocal increase in any measured lesion or the appearance of new lesions. Patients were defined as having an "early death" if death occurred within the first 14 days of therapy. All reported responses must have occurred outside concurrently treated radiation therapy portals.

DEMOGRAPHIC DATA

Thirty-six patients were entered in the study, 30 with adenocarcinoma and six with large cell carcinoma. There were 20 males and 16 females with a median age of 58 years (range, 36–80) and a median Karnofsky performance status of 70% (range, 40%–100%). In the adenocarcinoma group, 11 patients had stage III M_0 disease and 19 had stage III M_1 disease. The large cell carcinoma group was composed of three patients with stage III M_0 disease and three with stage III M_1 disease. In the adenocarcinoma group, three patients had been previously treated with chemotherapy (methotrexate plus methyl-CCNU) and had progressive disease at the time of entry in the study. Eleven patients had been previously irradiated. No previous therapy, except surgery, had been administered to patients with large cell carcinoma. Two patients with adenocarcinoma were considered to be nonevaluable because of early death, and one patient with large cell carcinoma was lost to followup and was also considered nonassessable.

RESULTS

Large Cell Carcinoma

No responses were seen in this subgroup.

Adenocarcinoma

Seven partial remissions, all in previously untreated patients, were observed in this subgroup of 28 evaluable patients, giving a response rate of 25%. Median duration of response was 14 weeks with a range of 6–37 weeks. One patient continues in remission at 37 weeks and one was lost to followup at 16 weeks.

Response showed no relationship with the degree of bone marrow suppression, with only one patient developing a wbc count of < 3000/mm^3. Median survival for responding patients will be at least 39 weeks, but has not yet been reached because four patients remain alive at 44, 45, 49, and 71 weeks. Median survival for nonresponders was 17 weeks, with two patients alive at 30 and 47 weeks (fig 1). This difference is statistically significant ($P < 0.004$). When comparing patients with stage III M_0 and stage III M_1 disease, no difference was observed in response rate (three of 11 patients and four of 19 patients) or in survival (22 and 21 weeks).

TOXICITY

Nonhematologic toxicity consisted of nausea (45%), vomiting (40%), stomatitis (5%), anorexia (5%), alopecia (60%), and increase in skin pigmentation (one patient).

Hematologic toxicity is shown in tables 1 and 2. Significant leukopenia (< 3000 cells/mm^3) was observed in 25% of the courses (20% of previously untreated and 70% of previously treated patients) and thrombocytopenia (< 100,000 cells/mm^3) was observed in only 5% of the courses. No cumulative myelotoxicity was noted, nor was any significant delay necessary in administering subsequent courses of therapy. There was no pulmonary or renal toxicity, and no toxic deaths occurred.

DISCUSSION

While ineffective in large cell carcinoma of the lung, the combination of mitomycin C, Adriamycin, and cyclophosphamide (MAC) in this phase II study showed rea-

TABLE 1.—Hematologic toxicity

	No. of courses	Wbc count			Platelet count		
		Nadir (cells × 10^3/mm^3)	Median days to—*		Nadir† (cells × 10^3/mm^3)	Median days to—*	
			Nadir	Recovery		Nadir	Recovery
Good-risk patients	20	Mean, 5.35 Median, 3.70 Range, 1.25–29	14	23	Mean, 208.60 Median, 206.50 Range, 63–510	20	24
Poor-risk patients	10	Mean, 3.44 Median, 3.75 Range, 1.10–6.5			Mean, 212.80 Median, 192.00 Range, 87–400		

*"Toxic" courses only (wbc count < 3000/mm^3 and platelet count < 100,000/mm^3).
†No thrombocytopenia noted.

TABLE 2.—Lowest blood cell counts

	No. of courses	No. of courses with grade—*				
		4	3	2	1	0
		Wbc count				
Good-risk patients	20	—	5	2	3	10
Poor-risk patients	10	—	3	1	3	3
		Platelet count				
Good-risk patients	20	—	—	2	2	16
Poor-risk patients	10	—	—	—	2	8

*Wbc count (cells × 10^3/mm^3): grade 4 = 0–0.999; grade 3 = 1.0–1.9; grade 2 = 2.0–2.9; grade 1 = 3.0–3.9; grade 0 = ⩾ 4.0. Platelet count (cells × 10^3/mm^3): grade 4 = 0–24.9; grade 3 = 25–49.9; grade 2 = 50–74.9; grade 1 = 75–99.9; grade 0 = ⩾ 100.

sonable activity in adenocarcinomas of this organ, with a response rate of 25%. Significant prolongation of survival of responders was observed (at least 39 weeks) when compared to nonresponders (17 weeks) (fig 1). This combination appeared to be well-tolerated with acceptable and manageable myelotoxicity, which was more prominent in previously chemotherapeutically treated patients. Gastrointestinal toxicity and alopecia were moderate.

A comparison was made between this study and our previous mitomycin C study. With regard to prognostic factors known to have significance in lung cancer, including age, prior radiation therapy and/or chemotherapy, performance status, and disease-free interval, both patient populations were comparable. Four differences were found that, in our opinion, do not affect comparability:

1. The dose of mitomycin C was reduced from 20 mg/m^2 to 5–7 mg/m^2.
2. More non-whites were entered in the MAC study.
3. More patients had undergone curative resections in the MAC study.
4. More patients treated with mitomycin C alone had extrapulmonary metastases.

Both studies demonstrated a significant survival advantage for responders compared to nonresponders, with a median survival of 33 versus 9 weeks for mitomycin C and at least 39 versus 17 weeks for MAC. While MAC appears to be superior in terms of overall duration of response (14 versus 6 weeks) and survival, both studies fell short of reaching statistical significance (fig 2). Toxicity was less marked in the MAC study, with no cumulative myelotoxicity, lung toxicity, or renal toxicity observed.

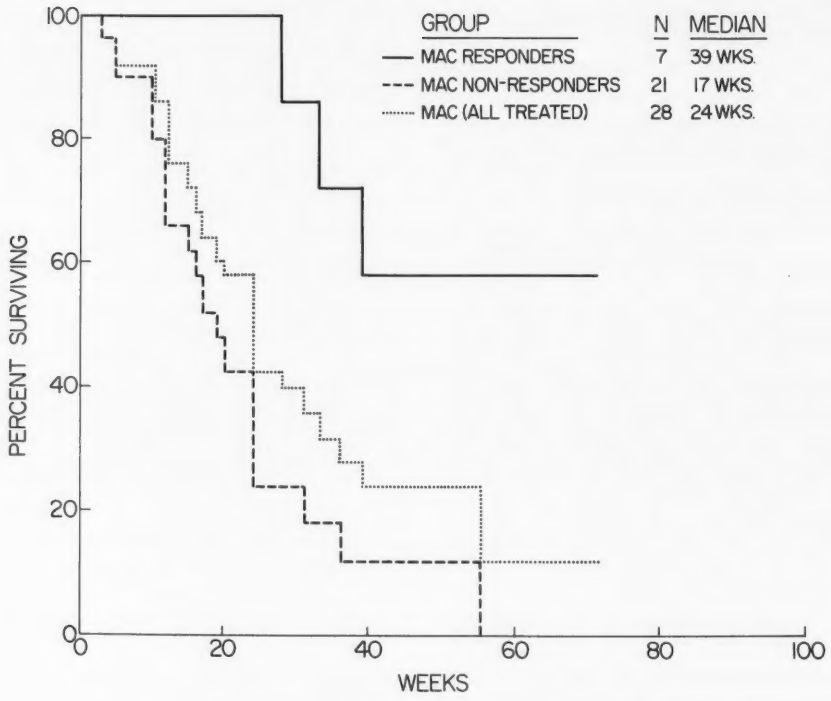

FIGURE 1.—Survival of patients treated with MAC.

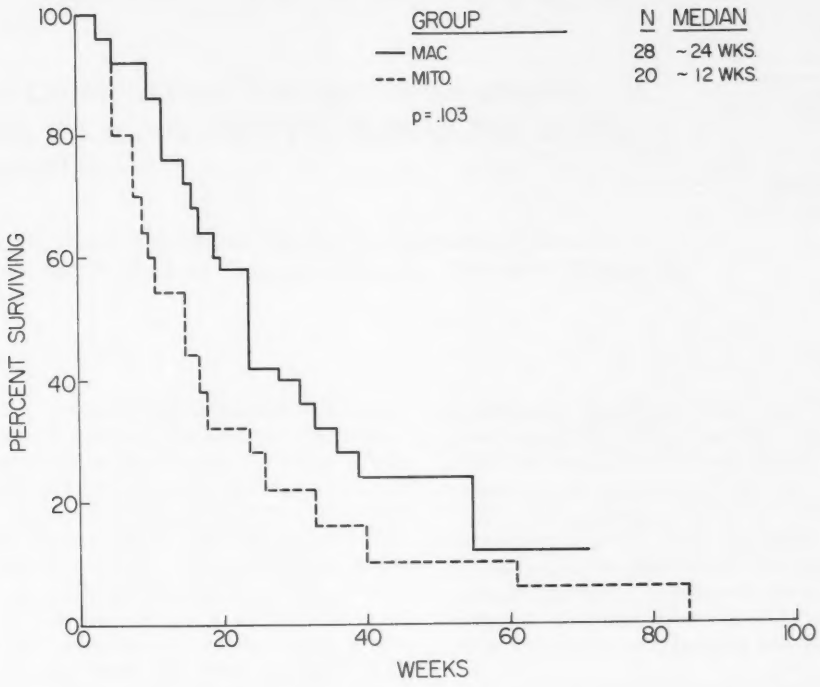

FIGURE 2.—Comparison of survival for patients treated with MAC vs patients treated with mitomycin C alone.

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DTIC and Combination Therapy for Melanoma. II. Escalating Schedules of DTIC With BCNU, CCNU, and Vincristine [1,2,3]

Brief Reports and Preliminary Communications

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SUMMARY

A total of 392 patients with metastatic malignant melanoma were entered in a randomized chemotherapy study conducted by the Central Oncology Group (COG) between 1974 and 1976 (COG protocols 7131 and 7131A). The results of therapy with DTIC alone were compared with those of DTIC and vincristine (VCR) plus either BCNU or CCNU. Progressive dose elevations of 20% were scheduled to produce toxic effects in patients who did not initially experience toxic effects or complete response. Of the 392 patients who were entered in the study, 337 were evaluable. The response rate was 20.2% for evaluable patients (68 of 337) and 17.3% for all patients (68 of 392) entered in the study. These results are similar to those reported in a previous COG study of DTIC alone and DTIC in combination with VCR and lower doses of BCNU and CCNU, in which responses occurred in 17.3% of evaluable patients (243) and in 15.5% of all patients (270) entered in the study (COG protocol 7130). The following conclusions were made: (*a*) the results showed no statistically significant differences in the response rates among the various treatment arms; (*b*) deliberate efforts to escalate to toxicity did not increase the response rate in patients treated with a combination of DTIC, VCR, and either BCNU or CCNU; (*c*) deliberate escalation of DTIC alone may have produced an increased response rate (up to 29%) but did not change median duration of response or survival; and (*d*) a wide range of tolerance to the toxicity of DTIC and the other drugs used in this study was found.

[Cancer Treat Rèp 63:1989–1992, 1979]

In a deliberate attempt to produce toxicity, Central Oncology Group (COG) protocol 7131 utilized 33%–67% higher initial drug doses in combinations than COG 7130 (1) used, since patients with a wbc count of $< 4000/mm^3$ had a 35% response rate compared to the nonleukopenic patients in the prior study with an 8% response rate.

COG 7131 utilized the drug combinations which had been determined in COG 7130 to be advantageous with respect to response, toxicity, and ease of administration. In evaluating the preliminary results of COG 7131 as they were received, it became apparent that an even higher escalation in the combinations would probably be needed to achieve toxicity promptly before serious tumor progression occurred. In COG 7131A, all initial drug doses, except the initial dose of DTIC alone, were increased by an additional 20%. In COG 7131A, patients were scheduled to receive 20% escalations in all drug doses in each succeeding course of therapy, until the patient achieved either a complete response or developed toxicity in the form of leukopenia (wbcs $< 4000/mm^3$), thrombocytopenia (platelets $< 125{,}000/mm^3$), or some other type of severe toxicity.

MATERIALS AND METHODS

Patient selection, evaluation procedures, and statistical methods in COG 7131 were identical to those used in

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[3]DTIC, BCNU, and CCNU were supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[4]This study was performed by the Central Oncology Group (protocol 7131; G. J. Hill II, chairman). The following investigators and institutions participated in the report: *G. J. Hill II*, Washington University, St. Louis, MO (*present address:* Marshall University, Huntington, WV); *G. E. Metter, G. Ramirez,* and *S. E. Moss*, University of Wisconsin, Madison; *E. T. Krementz*, Tulane University, New Orleans, LA; *W. S. Fletcher*, University of Oregon, Portland; *F. M. Golomb*, New York University Medical Center, New York; and *T. B. Grage*, University of Minnesota, Minneapolis.

[5]We are grateful to Kathleen E. Rebstock for data management and evaluation.

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TABLE 1.—Treatment plan

COG 7131			COG 7131A			
Treatment arm	Dose (mg/kg)	Days	Treatment arm	Dose (mg/kg)*	Days	Courses repeated—
DTIC	4.5	1–10	DTIC	4.5	1–10	Every 4 wks
DTIC	2.7	1–5	DTIC	3.2	1–5	Every 6 wks
CCNU	2.0	2	CCNU	2.4	2	
VCR	0.027	1 and 5	VCR	0.032	1 and 5	
DTIC	2.7	1–5	DTIC	3.2	1–5	Every 6 wks
BCNU	3.0	2	BCNU	3.6	2	
VCR	0.027	1 and 5	VCR	0.032	1 and 5	
DTIC	4.5	1–5	DTIC	5.4	1–5	Every 6 wks
BCNU	2.0	2	BCNU	2.4	2	
VCR	0.027	1 and 5	VCR	0.032	1 and 5	

*COG 7131A doses increased by 20% in each subsequent course.

COG 7130. Patients who had previously received DTIC, or those who were randomized to receive DTIC alone initially, were eligible to receive one of the combination therapy programs after disease progression with DTIC. Dose schedules for COG 7131 are shown in table 1. In COG 7131A, DTIC and all other drugs except vincristine (VCR) were increased by 20% in each subsequent course.

RESULTS

A total of 392 patients were entered in this study and all have been evaluated; 337 (84.0%) are considered evaluable and 55 (16%) are considered unevaluable. Of a total of 235 patients treated in COG 7131, 212 (90%) were evaluable and of a total of 157 patients treated in COG 7131A, 125 (80%) were evaluable. Patients were grouped into two prior treatment categories: evaluable patients with no prior DTIC therapy (169 in COG 7131 and 96 in COG 7131A) and evaluable patients who had previously received DTIC therapy (43 in COG 7131 and 29 in COG 7131A). The 55 unevaluable patients were evenly distributed among the treatment arms.

Response

Responses occurred in 68 patients in COG 7131 and 7131A; this represents a response rate of 17.3% among the 392 study patients and 20.2% among the 337 evaluable patients. Responses occurred in 36 (17.0%) of the 212 evaluable patients in COG 7131 and in 32 (25.6%) of the

TABLE 2.—Response rates for evaluable patients

	Patient responses									
	Complete		Partial		No change		Progression		Total†	
Treatment*	No.	%	No.	%	No.	%	No.	%	No.	%
COG 7131										
DTIC	2	3	8	13	24	40	26	43	60	99
DTIC, CCNU, and VCR	3	6	6	12	18	36	23	46	50	100
DTIC (2.7), BCNU (3.0), and VCR	2	4	5	11	27	59	12	26	46	100
DTIC (4.5), BCNU (2.0), and VCR	2	4	8	14	31	55	15	27	56	100
Totals	9	4	27	13	100	47	76	36	212	100
COG 7131A										
DTIC	2	6	8	23	15	43	10	29	35	101
DTIC, CCNU, and VCR	2	6	5	14	21	58	8	22	36	100
DTIC (3.2), BCNU (3.6), and VCR	0	0	6	21	19	66	4	14	29	101
DTIC (5.4), BCNU (2.4), and VCR	3	12	6	24	9	36	7	28	25	100
Totals	7	6	25	20	64	51	29	33	125	100

*Nos. in parentheses = dose in mg/kg.
†Variations in percentages due to rounding error.

TABLE 3.—Toxicity

Toxic effect and treatment*	Initial doses: % of patients, Toxicity grade 0	1	2	3	4	Total No. of patients	20% escalation: % of patients, Toxicity grade 0	1	2	3	4	Total No. of patients
						COG 7131						
Nausea and vomiting												
DTIC	2	39	40	16	4	57	20	20	40	20	0	5
DTIC, CCNU, and VCR	18	42	28	12	0	50	20	20	20	40	0	5
DTIC (2.7), BCNU, and VCR	17	35	37	9	2	46	50	0	50	0	0	2
DTIC (4.5), BCNU, and VCR	13	26	43	18	0	53	11	22	67	0	0	9
Leukopenia												
DTIC	75	10	9	4	2	57	60	20	0	0	20	5
DTIC, CCNU, and VCR	54	20	10	16	0	50	40	40	20	0	0	5
DTIC (2.7), BCNU, and VCR	56	24	15	4	0	46	100	0	0	0	0	2
DTIC (4.5), BCNU, and VCR	51	26	17	6	0	53	22	56	11	0	11	9
Thrombocytopenia												
DTIC	77	10	2	2	9	57	80	0	0	0	20	5
DTIC, CCNU, and VCR	70	8	8	12	2	50	60	0	20	20	0	5
DTIC (2.7), BCNU, and VCR	78	15	4	2	0	46	100	0	0	0	0	2
DTIC (4.5), BCNU, and VCR	62	13	11	6	8	53	56	22	0	0	22	9
						COG 7131A						
Nausea and vomiting												
DTIC	18	21	45	9	6	33	11	11	56	22	0	19
DTIC, CCNU, and VCR	17	14	47	22	0	36	7	43	36	14	0	14
DTIC (3.2), BCNU, and VCR	14	18	32	36	0	28	25	0	25	38	12	8
DTIC (5.4), BCNU, and VCR	17	12	33	21	17	34	0	10	70	20	0	10
Leukopenia												
DTIC	67	12	12	9	0	33	68	21	0	11	0	19
DTIC, CCNU, and VCR	53	28	14	6	0	36	29	43	29	0	0	14
DTIC (3.2), BCNU, and VCR	43	14	36	4	4	28	62	0	25	12	0	8
DTIC (5.4), BCNU, and VCR	38	29	29	4	0	24	20	30	50	0	0	10
Thrombocytopenia												
DTIC	73	6	3	6	12	33	74	11	0	5	11	19
DTIC, CCNU, and VCR	69	6	11	8	6	36	57	7	21	7	7	14
DTIC (3.2), BCNU, and VCR	54	14	4	4	25	28	62	0	12	12	12	8
DTIC (5.4), BCNU, and VCR	50	4	25	8	12	24	70	26	10	0	0	10

*Nos. in parentheses = dose in mg/kg.

125 evaluable patients in COG 7131A (table 2). Forty-eight patients treated previously with DTIC were randomized to one of the three combination therapy arms following progression. There were 11 responses (ten partial and one complete [23%]) with DTIC combination therapy. On the other hand, there were no responses among 24 patients previously treated with a DTIC combination and then treated with DTIC alone. Responses occurred in 57 (21.5%) of the 265 evaluable patients who had received no prior chemotherapy, whereas there were only 11 (15.3%) responses among the 72 previously treated patients. Median durations of response ranged from 11.0 to 31.9 weeks. The differences between treatments are not significant, and no significant improvement occurred with dose escalation.

Patients exhibiting leukopenia in COG 7131 had a 24% response rate compared to patients without leukopenia, who had a 9% response rate; this is a statistically significant difference ($P < 0.001$). In COG 7131A, the similar response rates were 34% and 8%, also statistically significant ($P < 0.005$). A similar analysis regarding thrombocytopenia produced no significant results in COG 7131, although in COG 7131A, the patients with thrombocytopenia responded better (42% versus 8%; $P < 0.0001$).

Survival

The median durations of survival ranged from 22.9 to 47.1 weeks. There was no statistically significant difference in survival among the treatment arms. In COG 7131, females survived slightly, but significantly, longer than males ($P = 0.046$), with a median survival time of 33 weeks, compared with a median survival time of 23 weeks for males.

Toxic Effects (table 3)

At the starting dose of COG 7131, nausea and vomiting occurred in 88% of patients, leukopenia was seen in 40%, and thrombocytopenia was seen in 23% (life-threatening severity in 5%). Over 80% of the patients in each treatment arm experienced some degree of nausea and vomiting; only one patient in the DTIC-alone treatment arm did not experience nausea and vomiting. Leukopenia was experienced by 25% of the patients receiving DTIC alone and by 46% of the patients in the combination treatment arms. Thrombocytopenia was experienced by 22%–38% of the patients in the various treatment arms; platelet counts of $< 50{,}000/mm^3$ were found in five of the patients (9%) receiving DTIC alone, in one receiving DTIC, CCNU, and VCR, and in four (8%) receiving DTIC (4.5 mg/kg), BCNU, and VCR. Other or unusual toxic effects occurring during the starting dose in COG 7131A were observed in 38 patients. The dose-limiting toxicity was difficult to define in many patients, being a combination of severe nausea and vomiting, fatigue, and malaise, coupled with a moderate degree of pancytopenia. Experienced chemotherapists found the progressive dose escalations difficult to administer, more often because of pernicious vomiting than leukopenia, which had been expected to be the dose-limiting form of toxicity.

In COG 7131A, a 20% escalation was carried out in 51 patients, a 40% escalation in 11, a 60% escalation in three, and an 80% escalation in two. At the 20% escalation, 83% of patients developed moderate or severe hematologic or gastrointestinal toxicity, and at each successive escalation, a higher percent of the patients experienced severe toxic effects (both hematologic and nonhematologic).

This study confirms the lack of significant clinical benefit observed in most patients treated with DTIC, including patients treated in the adjuvant setting where survival may actually be worse, except in patients who develop hematologic toxic effects (2).

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Phase I Study of Anguidine Administered Weekly [1]

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SUMMARY

Anguidine was administered on a weekly schedule to 20 patients. Gastrointestinal and neurologic toxic effects were dose-limiting for both 4- and 8-hour infusions of anguidine. Myelosuppression was infrequent and not dose-related. Recommended starting doses for weekly anguidine are 6.0 mg/m² for patients with normal hepatic function and 3.5 mg/m² for patients with liver dysfunction.

[Cancer Treat Rep 63:1993–1995, 1979]

Anguidine (diacetoxyscirpenal) is a phytotoxic product of the fungus, *Fusarium equiseti*, initially selected for phase I studies in man based on its activity against the P388 animal tumor system.[6] Previous phase I trials of anguidine evaluated daily × 5 schedules (1,2). We report here our results of a phase I study of anguidine administered by a weekly schedule.

MATERIALS AND METHODS

Anguidine was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD), in 5-mg vials. The drug was dissolved in sterile water and further diluted in 500 ml of 5% dextrose in water. All patients treated had advanced malignancies resistant to conventional therapeutic modalities; they had given informed consent and had not received chemotherapy or radiotherapy for at least 3 weeks (6 weeks for nitrosoureas and mitomycin C) prior to treatment with anguidine. A total of 20 patients received 33 courses of anguidine administered by a weekly schedule. Courses ranged from 1 to 19 weeks (median, 4 weeks) in duration. Seventeen patients (29 courses) received anguidine by 4-hour infusion, and three patients (four courses) were treated with 8-hour infusions. The 4-hour infusion time was selected because we had previously demonstrated that infusions of anguidine significantly reduced the hypotension and neurotoxicity associated with bolus administration, and that 4-hour infusions reduced the incidence of severe myelosuppression seen with 8-hour infusions (1). Eight-hour infusions were given to a few patients at the higher dose levels to determine if dose-limiting myelosuppression could be achieved with the weekly schedule as had been previously demonstrated with the daily × 5 schedule. The starting dose of anguidine was 2.0 mg/m² and dose escalations of 0.5–1.0 mg/m² were made up to a maximum dose of 10 mg/m². Three patients received 0.5–1.0-mg/m² incremental increases in doses provided that no toxicity was encountered after 4 weeks of treatment at the lower dose.

All patients had solid tumors and had received previous chemotherapy. In addition, nine of 20 patients had been treated with radiotherapy. Eight patients had liver metastases demonstrated by filling defects on radionuclide scans of the liver. Only one patient with documented liver metastases received a dose > 4.0 mg/m² because of dose-limiting toxicity and previous knowledge that liver dysfunction increases drug toxicity (1).[7] Patients continued to receive treatment unless there was disease progression or prohibitive toxicity.

RESULTS

Drug-related toxicity is shown in tables 1 and 2. Hematologic toxicity was uncommon with both the 4- and 8-hour infusion schedules. Leukopenia was seen in only two patients and did not appear to be dose-related. In one patient, the degree of myelosuppression did not

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[5]We gratefully acknowledge the help of Lyn Cain, RN, and Rosemary Grady, RN.

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[7]Bodey GP. Minutes of the Phase I Working Group Meeting, Oct 7, 1976.

TABLE 1.—Hematologic toxicity

	No. of patients receiving anguidine doses (mg/m²/wk) of—		
	2.0–3.5 (10 patients)	4.0–6.0* (16 patients)	6.5–10.0† (7 patients)
Leukopenia (wbcs/μl)			
> 4000	9	12	6
3000–4000	—	3	1
2000–3000	1	1	—
< 2000	—	—	—
Thrombocytopenia (platelets/μl)			
> 100,000	5	15	5
75,000–100,000	2	1	2
50,000–75,000	2	—	—
25,000–50,000	1	—	—

*Includes 1 course of anguidine by 8-hr infusion.
†Includes 3 courses of anguidine by 8-hr infusion.

TABLE 2.—Nonhematologic toxicity

	No. of patients receiving anguidine doses (mg/m²/wk) of—		
	2.0–3.5 (10 patients)	4.0–6.0* (16 patients)	6.5–10.0† (7 patients)
Nausea and vomiting			
None	1	2	—
Mild	3	3	1
Moderate	6	10	2
Severe	—	1	4
Neurologic‡			
None	7	14	1
Mild	3	2	3
Moderate	—	—	3
Severe	—	—	—
Fever/chills	4	4	4

*Includes 1 course of anguidine by 8-hr infusion.
†Includes 3 courses of anguidine by 8-hr infusion.
‡Grading of neurologic toxicity: mild = drowsiness, headaches, or anxiety; moderate = depression or confusion; and severe = coma, psychosis, seizures, or hallucinations.

increase despite escalation of the dose from 4.0 to 6.0 mg/m². Thrombocytopenia was unpredictable, and was only seen in patients with prior nitrosourea therapy.

Gastrointestinal and neurologic toxic effects were dose-limiting at doses > 4.0 mg/m² for patients with liver dysfunction (liver metastases and/or elevated bilirubin, SGOT, and alkaline phosphatase) and at doses > 6.0 mg/m² for patients without liver dysfunction. Gastrointestinal toxicity was primarily nausea and vomiting although an occasional patient had diarrhea. Neurologic dysfunction included dizziness, orthostatic hypotension, and headaches. In addition, a neurasthenic syndrome of fatigue, malaise, and depression was common and at times disabling in patients receiving doses > 3.5 mg/m² and was universal in patients treated at the 8.0- and 10.0-mg/m² dose levels with both the 4- and 8-hour infusion schedules. Two patients required dose reduction of anguidine due to gastrointestinal and neurologic toxicity.

Myelosuppression was seen in only one of the three patients treated with 8-hour infusions. Gastrointestinal toxicity was somewhat decreased compared to 4-hour infusions, but neurotoxicity was just as severe and dose-limiting. Neither renal nor hepatic toxicity was seen in this study. There were no drug-related deaths.

There were no responses seen in the 20 patients treated with weekly anguidine. Four of nine patients with colorectal cancer had stable disease (< 50% regression or 25% increase in measurable tumor) lasting 16–39 weeks (me-

dian, 23 weeks). All other patients had increasing disease.

DISCUSSION

Anguidine was given by weekly iv infusion to 20 patients with advanced malignancies. Neurologic and gastrointestinal toxic effects were dose-limiting for both 4- and 8-hour weekly infusions. Both gastrointestinal and neurologic toxic effects were dose-related and possibly more severe in patients with hepatic dysfunction. Hematologic toxicity was uncommon and not dose-limiting with the weekly infusion schedule. This is in contrast to the often severe myelosuppression seen with the 8-hour infusions given by a daily × 5 schedule (1,2). Cumulative toxicity was not seen in any patient treated with more than one weekly dose, including five patients who received 11–36 weekly injections of anguidine.

Anguidine has been reported to be synergistic with 5-fluorouracil in a colon xenograft system.[8] The stable disease seen in four patients with colorectal cancer suggests a degree of activity in this chemotherapeutic-resistant disease. However, a recent study combining 5-fluorouracil and anguidine using a 5-day schedule in the treatment of colorectal cancer produced only a 9% partial response rate.[9] Although possible, it is unlikely that a different schedule would produce significantly greater antitumor activity.

The absence of myelosuppression from anguidine with the weekly schedule would allow for combination therapy with myelotoxic agents. The recommended starting dose for weekly anguidine is 6.0 mg/m^2 administered by 4-hour infusion. In patients with liver metastases and/or hepatic dysfunction treatment should start at 3.5 mg/m^2.

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Combination Chemotherapy With Vincristine, Bleomycin, and Methotrexate for Advanced Head and Neck Cancers [1]

Brief Reports and Preliminary Communications

R. L. Woods,* J. Stewart, R. M. Fox, and M. H. N. Tattersall [2,3]

SUMMARY

Thirty-three patients with a histologically confirmed diagnosis of advanced squamous cell carcinoma of the head and neck were treated with a combination of vincristine, methotrexate, and bleomycin. The regimen was as follows: Day 1, vincristine (2 mg iv) and methotrexate (200 mg/m^2 iv over 24 hours); Day 2, bleomycin (15 mg/day by iv infusion for 48 hours). Folinic acid rescue began 36 hours after the start of methotrexate. The regimen was repeated at intervals of 3 weeks. Toxicity was minimal but the overall response rate was only 24% (three complete responses and five partial responses) and the median duration of remission was only 16 weeks overall. The combination of vincristine, methotrexate, and bleomycin in this dosage schedule has a response rate lower than that reported with single-agent treatment of advanced head and neck cancers.

[Cancer Treat Rep 63:1997–1999, 1979]

Head and neck cancers constitute approximately 5% of registered cancers in Western countries. Initial treatment is usually with radiotherapy or surgery since these tumors commonly present as local problems and only rarely metastasize widely. Cytotoxic chemotherapy has been evaluated in patients with recurrent local disease and in patients with advanced local tumors not suitable for treatment with surgery or radiotherapy. Several drugs have been reported to cause tumor regression, and methotrexate, the most extensively studied drug, has been used both iv and intra-arterially with an overall response rate of approximately 40% (1). The reported response rate with bleomycin is 31% although many of these were "minor" responses (< 50% reduction in tumor size) and the true partial response rate (> 50% reduction in tumor size) is 15% (2). There is little information on the activity of vincristine as a single agent (3). In vitro, vincristine combined with methotrexate may increase the intracellular concentration of methotrexate in cells, possibly by inhibiting cellular methotrexate efflux, but the results in different cell lines are not consistent (4,5). We now report the use of a regimen of vincristine, bleomycin, and methotrexate in a series of patients with histologically confirmed and measurable squamous cell carcinomas of the head and neck.

MATERIALS AND METHODS

Thirty-three patients were studied, of whom 28 were male and five were female. Their ages ranged from 41 to 80 years with a mean of 61 years. All patients had a histologic diagnosis of squamous cell carcinoma. Twenty-one patients had been previously treated with both surgery and radiotherapy, six patients had received radiotherapy only, and two patients had been treated with surgery only. Four patients had received no previous therapy for malignant disease. None of the patients were considered suitable for treatment with radiotherapy or surgery and all patients had measurable disease. No patient had any serious intercurrent disease. Pretreatment assessment included a complete history and physical examination, full blood cell count, 20-channel biochemical screening profile including serum creatinine, chest roentgenogram, and local roentgenogram. Patients with elevated serum creatinine concentrations (> 1.2 mg/100 ml) were excluded from the study. Prior to each course of chemotherapy, all patients had full clinical assessment including hematologic and biochemical measurements.

Chemotherapy was given according to the following schedule: Day 1, vincristine (2 mg total dose) as an iv bolus and methotrexate (200 mg/m^2), one third as an iv bolus and two thirds as infusion for 24 hours; Days 2 and 3, bleomycin (15 mg/24 hours) by iv infusion. Folinic acid

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[3] We are grateful to the surgical and radiotherapeutic members of the Head and Neck Oncology Section at the Royal Prince Alfred Hospital for their help and advice in the management of these patients.

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rescue (15 mg orally every 6 hours for 12 doses) was started 36 hours after methotrexate was given. Each course was repeated on Day 21.

A complete response (CR) was defined as disappearance of all subjective and objective evidence of tumor. A partial response (PR) was defined as a > 50% reduction in the sum of the products of the largest perpendicular diameters of the tumors. Any change of < 50% was considered a nonresponse. The duration of response was measured from the start of chemotherapy.

RESULTS

The distribution of primary tumor sites among the 33 patients is shown in table 1. There were three CRs (durations, 10, 12+, and 35+ weeks) and five PRs. The durations of the PRs were 4, 18, 26, and 35 weeks, with one responding patient lost to followup after 12 weeks. The responses according to the site of the primary lesion are also shown in table 1.

It is notable that two of the four patients who had no previous therapy responded, compared with six responses among the 29 patients who had had previous therapy. Three of the five females responded to treatment compared to five of the 28 males. The median survival duration overall, from start of chemotherapy, was 16 weeks. The median survival of the partial and complete responders has not yet been reached and is in excess of 28 weeks.

The toxicity of this regimen was minimal. Only one case of severe leukopenia (wbc count < 1000/μl) occurred and six patients developed moderate leukopenia (wbc count 2500–4000/μl). There were no cases of impairment of renal or hepatic function, mucositis, peripheral neuropathy, or pulmonary toxicity.

TABLE 1.—Distribution of primary tumor sites and responses according to site

	No. of patients	No. of responses	
		PR	CR
Tongue	9		1
Larynx	6		1
Buccal mucosa	4	1	1
Epiglottis	1		
Parotid	2		
Skin	5	1	
Fauces	2	1	
Lip	1		
Floor of mouth	1		
Tonsil	2	2	

DISCUSSION

The overall response rate of 24% with this regimen is less than that reported for methotrexate as a single agent. The high proportion of extensive lesions and the high incidence of previous therapy in this patient group may account for the low response rate observed. Widely differing response rates have been reported for patients with head and neck cancers using identical chemotherapy regimens. These differing reports may, in part, reflect the difficulty of assessing changes in tumor size in this region. Although many of the tumors are accessible, the previous radiotherapy which many of these patients have had often leaves marked induration, which can make true changes in tumor size difficult to assess. The results in this study were assessed by an independent observer.

One other possible reason for the disappointing results is that the drugs may have been given in suboptimal doses and the lack of toxicity may reflect this fact. Pouillart and Mathé (6) have reported a combination of vincristine, bleomycin, and methotrexate used in a similar group of patients but with a somewhat different schedule. The schedule used by these investigators was as follows: Day 1, vincristine (1.2 mg/m^2); Day 2, bleomycin (10 mg/m^2) and methotrexate (10 mg/m^2 × 4); and Day 3, bleomycin (10 mg/m^2) and methotrexate (10 mg/m^2 × 4). Nineteen of 21 patients responded with four CRs and 15 PRs, but there is no mention of the toxicity of the treatment. The similarity between the two regimens is clear, yet there is an enormous difference in response rates. It is useful to compare survival results between the two schedules to see if the patient populations were similar. In the study by Pouillart and Mathé, in spite of the high response rate, the mean survival time was only 10 weeks. This emphasizes the importance of measuring response and reporting survival time in those patients where accurate tumor measurement may be difficult.

Clifford (7) has recently reported the use of vincristine, methotrexate, and bleomycin in combination with other treatment modalities in a series of patients with squamous cell carcinoma of the head and neck. Their results are far superior to the results reported here. However, their patients received chemotherapy as part of their initial treatment rather than following relapse, and this may account for the different response rates. Their claim, however, that chemotherapy had a major impact on survival is difficult to equate with the low response rate the drug regimen had in advanced disease.

The treatment regimen reported here is not only disappointing in terms of overall objective tumor response rates, but also extremely costly in terms of the use of hospital beds. We conclude that this treatment program is not a significant advance compared to treatment by single agents.

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Neurologic and Cardiac Rhythm Abnormalities Associated With 4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (AMSA) Therapy [1,2]

Brief Reports and Preliminary Communications

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4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (AMSA) is a new antitumor drug that has recently been introduced into clinical trials. In phase I studies, the major toxic effect of AMSA was myelosuppression (1,2). Nonmyelosuppressive toxic effects were mild nausea or vomiting and/or phlebitis, which were observed in < 20% of the patients. After the phase I experience, phase II studies in various tumor types were initiated and approximately 120 patients with solid tumors and 30 patients with acute leukemia have been treated with this drug over the past 6 months at this institution.

The maximum-tolerated dose of AMSA for solid tumor patients is 40 mg/m^2/day × 3 days at 21-day intervals. Patients with leukemia were treated with AMSA at escalating doses of 50–120 mg/m^2/day × 3 days. More recently, the schedule of AMSA therapy for leukemia patients was modified to give the drug over 5–7 days using a daily dose of 75–90 mg/m^2. AMSA was administered iv in 250–500 ml of dextrose in water over a period of 1 hour. During the phase II studies we have reaffirmed the toxic effects observed in the phase I study. However, we unexpectedly observed generalized seizures in four patients and ventricular fibrillation in two, temporally related to AMSA infusion, which prompted this report.

CASE REPORTS

Patient 1

A 19-year-old female with acute leukemia previously treated with cytosine arabinoside and rubidazone received her first course of AMSA at a dose of 90 mg/m^2/day × 3 days. Because of poor response to the first course of AMSA she was given her second course at a dose of 90 mg/m^2/day × 5 days. The total AMSA dose of 125 mg dissolved in 250 ml of dextrose was given over 1 hour. On the third day, while receiving AMSA, the patient had a generalized seizure accompanied by loss of consciousness and urinary incontinence. She recovered after a short postictal phase. Neurologic evaluation revealed no abnormalities in her EEG, brain scan, and spinal fluid examination. The patient had no acid-base or electrolyte abnormalities. Because the etiology of her seizure was uncertain, she continued receiving AMSA to complete the 5-day course of therapy, without any further neurologic disturbance. Because of progressive leukemia, the patient subsequently received 3-deazauridine without response and died 10 days later. Examination of brain and meninges at autopsy was unremarkable. Although the patient was not known to be epileptic, she had a past history of one similar episode while receiving miconazole 5 months earlier.

Patient 2

A 44-year-old female with metastatic breast carcinoma involving bones and liver started receiving AMSA after failing to respond to 5-fluorouracil, Adriamycin, cyclophosphamide, and methotrexate. During the first course of AMSA given at a dose of 30 mg/m^2/day × 3 (50 mg/day), she experienced dizziness and blurred vision. Four weeks later, a second course of AMSA was started using the same dose. After two doses, and prior to receiving her third dose of the second course, she was found to be confused, drowsy, and unable to walk. Before she received her third dose the patient had a generalized seizure; therapy was discontinued. A complete neurologic evaluation, including brain scan, computerized tomogram, EEG, and spinal fluid examination, was normal. She recovered from this episode, but died of progressive disease 4 weeks later. Autopsy was not permitted by the family. This patient had had no prior history of epilepsy. However, she was known to have had idiopathic hypoparathyroidism, and her serum calcium generally remained at a level of 8–8.5 mg/dl but she had no symptoms

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or signs of tetany. Her serum calcium at the time of seizure was unchanged.

Patient 3

A 46-year-old male with acute leukemia that recurred after he had received multiple chemotherapeutic regimens, including rubidazone, was given AMSA. He tolerated the first course of 50 mg/m^2/day × 3 days, and 4 weeks later he started receiving a second course of AMSA at a dose of 75 mg/m^2/day × 3 days. On the second day, while receiving AMSA, the patient had a generalized seizure from which he recovered. He had had a craniotomy for evacuation of a traumatic subdural hematoma 3 weeks before he began the first course of AMSA. His seizure was felt to be posttraumatic and therapy was continued. In spite of treatment with Dilantin, he continued to have minor seizures for the next 48 hours. Three weeks later he was given a third course of AMSA at 90 mg/m^2/day × 3 without any further seizure activity. He developed problems of fluid retention and congestive heart failure, and after the third dose of AMSA he had an episode of supraventricular tachycardia which responded to digoxin and diuretics. The patient died 4 weeks later from multiple organ failure. No autopsy was done.

Patient 4

A 40-year-old male with acute leukemia who relapsed while receiving anthracyclines and cytosine arabinoside was given AMSA at a dose of 90 mg/m^2/day × 3 days. He tolerated the first course well and 3 weeks later began the second course at the same dose. While receiving the first dose of the second course he became dizzy and felt as though he was going to "black out." This was soon followed by a generalized seizure and a respiratory and cardiac arrest. Resuscitative efforts were begun and the cardiac monitor revealed the patient's rhythm to be ventricular fibrillation. After cardioversion he required ventilatory assistance and did well for 24 hours. On the third day he had repeated episodes of supraventricular and ventricular arrhythmias and then lapsed into coma. A brain scan and EEG were normal. ECG revealed no evidence of cardiac ischemia. Electrolytes and acid-base balance were within normal limits, except for a serum potassium value of 2.3 mEq/liter, which was rapidly corrected. He continued to deteriorate and died of sepsis 1 week later. Examination of the heart and the brain at autopsy revealed no abnormalities.

Patient 5

A 55-year-old female with acute leukemia previously treated with cytosine arabinoside and anthracyclines started receiving AMSA at a dose of 90 mg/m^2/day × 3 days on June 10, 1978. Seventeen days later a second course of AMSA was initiated at the same dose. While receiving the first dose of this course the patient had syncope and the cardiac monitor revealed ventricular fibrillation which responded to defibrillation; AMSA was continued. After the second dose the patient had an episode of supraventricular tachycardia and further AMSA therapy was discontinued. Associated medical conditions included an interstitial pneumonia with resultant hypoxemia, hyponatremia (Na = 118 mEq/liter), and hypokalemia (K = 1.9 mEq/liter). Her condition continued to deteriorate; she died of gram-negative bacteremia 4 weeks later. Autopsy examination was refused by the family.

DISCUSSION

Administration of single lethal doses of AMSA in dogs and monkeys has been reported to cause ataxia and seizures.[5] The basic mechanism of these nervous system derangements is not clear. Certainly, AMSA does not enter the brain in mice and rats as shown by Cysyk et al (3). Similarly, we were unable to detect AMSA in the cerebrospinal fluid from two patients who developed seizures.

Although no neurologic derangements were noticed during the phase I studies of AMSA, development of generalized seizures in four patients in a short period of time and in temporal relationship to iv infusion of AMSA makes us suspect that AMSA was the contributory factor to these episodes. However, it should be emphasized that all four patients had advanced disease and had associated medical conditions or complications that could certainly have been the contributory factors. Interestingly, all four patients were receiving the second course of AMSA when they developed seizures. Moreover, all patients except patient 2 had acute leukemia and received doses of AMSA that were twice as high as the maximum-tolerated dose among the solid tumor patients. None of the patients who developed seizures were receiving drugs which are known to cause seizures. All of these patients received AMSA in the standard dilution and over the prescribed period of time.

Although there is no clear evidence from animal studies that AMSA is cardiotoxic, two patients developed ventricular fibrillation during or after AMSA infusion. Once again, both patients had medical conditions such as hypoxemia and hypokalemia that could have contributed to the cardiac arrhythmias.

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[5]Henry MC, Port CD, Guarino AM, et al. Preclinical toxicologic evaluation of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide monochloride in mice, dogs, and monkeys. Report No. IITRI-Tox 249992-76-2, National Cancer Institute, National Institutes of Health (Bethesda, MD), Oct 20, 1976.

Following these incidents in the initial group of 15 patients with leukemia, AMSA administration has been carried out under careful cardiac monitoring, during drug infusion, and we have not observed any cardiac rhythm disturbances since then. In addition, EEGs done during AMSA infusion in five consecutive patients did not show any changes in cerebral cortical activity. Although initially we considered the larger doses used for leukemia patients as one possible factor related to these side effects, more recently we have been using much higher doses (90 mg/m^2/day $\times$ 7), and no neurologic or cardiac disturbances have been observed in the last 15 leukemia patients. Thus, although AMSA could be responsible for the neurologic and cardiac disturbances described in these patients, the causal role of AMSA has not been established. Our experience should, however, warrant caution and careful observation of patients when they are receiving AMSA, especially at doses of $\geq$ 90 mg/m^2/day. Since the electrolyte abnormalities could have been the predisposing factors to these episodes, careful monitoring and correction of any metabolic disturbances are essential before AMSA is administered.

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cis-Dichlorodiammineplatinum(II) Alone and Combined With DTIC for Treatment of Disseminated Malignant Melanoma [1,2,3]

Brief Reports and Preliminary Communications

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SUMMARY

Twenty-one patients with disseminated malignant melanoma were treated with *cis*-dichlorodiammineplatinum(II) (*cis*-DDP): ten with *cis*-DDP alone at a dose of 20 mg/m² iv × 4 days every 4 weeks and 11 with *cis*-DDP at a dose of 15 mg/m² iv × 4 days combined with DTIC at a dose of 200 mg/m² iv × 5 days every 4 weeks. Two of 21 patients (10%) showed a partial response to therapy. There were no complete responses and no apparent increases in length of survival for responders compared to nonresponders. One drug-related death occurred. Otherwise, drug toxicity was acceptable and appeared to be diminished by iv hydration and prolonged administration. In this study, *cis*-DDP was not effective for treatment of disseminated malignant melanoma.

[Cancer Treat Rep 63:2005–2007, 1979]

Therapy for disseminated malignant melanoma is unsatisfactory. Response rates to currently available anticancer drugs are low (1) and significant increases in length of survival are unusual (1,2). DTIC is a standard agent for therapy for disseminated malignant melanoma when given alone or in combination with other drugs, with disease regression being reported in 15%–42% of the patients so treated (although the higher figures are unusual) (1,2). *cis*-Dichlorodiammineplatinum(II) (*cis*-DDP) is an investigational agent that has been highly effective therapy for disseminated solid tumors including testicular neoplasms, ovarian cancer, and bladder cancer (3). The mechanism of action of *cis*-DDP and DTIC is thought to be similar (4,5), but the toxicity of the two drugs differs (3). Because of the success of *cis*-DDP against other solid tumors, a trial of this drug alone and in combination with DTIC for the treatment of disseminated malignant melanoma was initiated.

MATERIALS AND METHODS

Patients

Twenty-one patients (15 males and six females, 13–67 years old) with documented stage III (disseminated) malignant melanoma were entered in the study which was closed October 31, 1978. All patients had measurable disease. The first ten patients entered were treated with *cis*-DDP alone; the next 11 patients received combination *cis*-DDP and DTIC. In the *cis*-DDP-alone group, five patients were treated for lung metastases, four for subcutaneous or nodal lesions, and one for liver metastases. In the *cis*-DDP-DTIC group, six patients were treated for lung metastases, two for subcutaneous or nodal lesions, two for liver metastases, and one for metastases to small bowel.

All patients were at least ambulatory and capable of light work at the initiation of therapy, and all were followed until disease progression or death. Patients who showed disease progression or who failed to respond to two courses of therapy were given alternative treatments. Informed consent was obtained from all participants in the study.

One patient in the *cis*-DDP group had had prior chemotherapy (DTIC), whereas four patients in the *cis*-DDP-DTIC group had previously received other drugs. (One patient had received high-dose methotrexate and citro-

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vorum rescue, two had received bleomycin, vincristine, DTIC, high-dose methotrexate, and citrovorum rescue, and one had received actinomycin D, vincristine, and DTIC.)

Treatment Protocol

Patients were hospitalized and hydrated with iv fluids prior to and during the course of *cis*-DDP therapy. In the *cis*-DDP-alone group, the dose was 20 mg/m^2/day × 4 days. The drug was administered in 2000 ml of 5% dextrose in normal saline given iv over 6–8 hours, plus 40 mg of furosemide and 37.5 g of mannitol. In the *cis*-DDP-DTIC group, the dose of *cis*-DDP was 15 mg/m^2 × 4 days given as above and the dose of DTIC was 200 mg/m^2/day × 5 days given iv during the same period as the *cis*-DDP. In both groups, drug therapy was given every 4 weeks. Audiometry and creatinine clearance were obtained before each treatment course. Complete response (CR) to therapy was defined as the disappearance of all manifestations of disease. Partial response (PR) was defined as a regression of ≥ 50% of the initial size of measurable lesions lasting at least 1 month.

RESULTS

Response to Therapy

Two of 21 patients (10%) in this study had PRs. There were no CRs. An additional patient in each treatment group had an apparent stabilization of disease.

The patient with PR who was treated with *cis*-DDP alone showed a > 50% decrease in the size of subcutaneous metastases lasting 5 months. Another patient receiving *cis*-DDP only had a small decrease in size of a large pulmonary nodule that remained stable until his death from metastases to the liver and brain 5 months later. In the *cis*-DDP-DTIC group, the patient with PR had complete regression of pulmonary lesions and marked (> 50%) regression of skin nodules lasting 6 months. A second patient in the *cis*-DDP-DTIC group had a minimal reduction in size of pulmonary metastases that remained stable for 4 months. None of these four patients had received prior chemotherapy. The responses were evident after the first treatment course. Response data are summarized in table 1.

The other 17 patients in the study showed no evidence of regression of lesions or other beneficial response to therapy; two of these patients received only one course of drug and died from rapidly progressing disease. One patient died from drug toxicity and will be discussed below.

Survival following initiation of *cis*-DDP therapy was 9 months for the patient who had a PR with *cis*-DDP alone. Survival was 7 months for the patient who had a PR in the *cis*-DDP-DTIC group. Median survival following initiation of therapy was 7 months (range, 2–16 months) for the other 19 patients. Twelve of the 20 patients who have died had brain metastases. There was no apparent survival benefit for patients responding to therapy.

TABLE 1.—Malignant melanoma: response to *cis*-DDP with or without DTIC

	cis-DDP	*cis*-DDP-DTIC
No. of patients	10	11
No. of responses (type)	1 (PR)	1 (PR)
Length of response (mos)	5	6
Survival (mos)		
Responders*	9	7
Nonresponders*		
Median	7	7
Range	2–16	1–11

*Survival after start of *cis*-DDP therapy.

Toxicity

Twenty of 21 patients treated showed no evidence of renal toxicity. One patient died in renal failure following a second course of therapy. One patient receiving five courses of *cis*-DDP-DTIC complained of mild hearing loss (confirmed on audiogram). In the other 20 patients treated, there was no change in audiometric findings or subjective sense of hearing, and no tinnitus. Nausea and vomiting were minimal following *cis*-DDP given alone by this protocol, but there were frequent complaints of nausea and vomiting following combined *cis*-DDP-DTIC. Bone marrow suppression following treatment with *cis*-DDP alone was minimal. In the *cis*-DDP-DTIC group, four patients had therapy delayed because of low blood counts. Two patients (one in each treatment group) had a fall in hemoglobin of > 1.5 g/100 ml that was not related to rapid disease progression. Therapy was discontinued in one patient in the *cis*-DDP-DTIC group because of myelosuppression.

Patient Death

An apparent drug-related death occurred in a 53-year-old male with pulmonary and bone metastases of malignant melanoma. During the first course of *cis*-DDP-DTIC, the patient developed a fever of 39°C and respiratory wheezing. The drug was stopped and symptoms cleared promptly. There was no evidence of hepatic, renal, or bone marrow toxicity. A second course of therapy was given 3 weeks later. After the second dose of *cis*-DDP, the patient developed severe respiratory wheezing, hypoxia, severe hypotension, and oliguria. The patient was resuscitated, but he developed severe renal

insufficiency, a steady rise in serum hepatocellular enzymes and bilirubin, confusion, and progressive pulmonary insufficiency; he died 6 days following the episode. Autopsy findings showed massive hepatic necrosis plus metastatic melanoma in the skull, kidneys, and both lungs. The patient's death appeared to be due to the initial episode of shock followed by hepatorenal failure.

DISCUSSION

In this study, the response rate in patients with disseminated malignant melanoma treated with *cis*-DDP alone or in combination with DTIC was no better than response rates for single-agent DTIC therapy (1,2). Survival was not extended by drug treatment in either group. These findings confirmed the low response rate of malignant melanoma to *cis*-DDP as observed in two other studies (6,7). The dose of *cis*-DDP employed in this trial is in the intermediate range but is adequate to produce a relatively high response rate in ovarian or bladder carcinoma (3), indicating that malignant melanoma is relatively insensitive to the drug at this dose. More than one half of the patients died with brain metastases. Both *cis*-DDP and DTIC penetrate the blood-brain barrier poorly, which may limit the ability of these drugs to prolong survival in this disease.

The toxicity of the dose schedule of *cis*-DDP was acceptable. The pretreatment hydration, induced diuresis, and extended time of administration reduced gastrointestinal symptoms and nephrotoxic effects from *cis*-DDP to a minimum. This finding has been noted by other investigators (6,8). The death occurring after *cis*-DDP-DTIC therapy appeared to be an untoward hepatic reaction either directly or indirectly attributable to the drugs. The timing of the reaction was inappropriate for an anaphylactic response but the clinical picture was compatible with this type of systemic hypersensitivity. Allergic responses to *cis*-DDP have been reported (9,10) and are a risk of therapy with this drug. Severe hepatic toxicity from *cis*-DDP has not been reported.

Because of the low response rate and lack of survival benefit from *cis*-DDP therapy observed in this study and other reported trials, it is concluded that this drug is not a useful agent for treatment of disseminated malignant melanoma.

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cis-Dichlorodiammineplatinum(II) and DTIC in Malignant Melanoma[1]

Brief Reports and Preliminary Communications

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SUMMARY

Twenty-nine patients with advanced malignant melanoma were randomized to receive DTIC at a dose of 200 mg/m² iv on Days 1–5 and *cis*-dichlorodiammineplatinum(II) at a dose of 40 mg/m² iv on Days 1 and 4, repeated every 4 weeks (group A), or the same drugs plus procarbazine at a dose of 75 mg/m² orally daily on Days 1–8 and vincristine at a dose of 1.4 mg/m² iv on Day 1 (group B). These drugs were generally well-tolerated, but five of 16 patients in group A and six of 13 patients in group B required dose modification for either hematologic or renal toxicity. There were six objective responses among 16 patients in group A including one complete regression, while there were two objective responses among 13 patients in group B.

[Cancer Treat Rep 63:2009–2010, 1979]

In disseminated malignant melanoma, the response rate to the more successful single agents such as DTIC and the nitrosoureas is about 20% (1). Numerous attempts have been made to increase the response rate by the combination of various drugs (2). However, there is no conclusive evidence as yet in a large series that any particular combination has improved these results.

Eighteen patients were treated at the Roswell Park Memorial Institute and 11 patients were treated at the Institute Nacional de Cancerologia in one of two treatment groups as detailed below.

MATERIALS AND METHODS

There were 29 patients, 12 males and 17 females, with a mean age of 49.8 years (range, 26–68). Twenty-one of these patients had received no prior therapy while eight had had previous treatment. At the time of initiation of their chemotherapy, 26 patients had widespread disease, while three had extensive regional recurrences. All of the 29 patients had measurable disease.

Sixteen patients were randomized to chemotherapy group A and 13 patients were randomized to group B. Treatment A consisted of DTIC (200 mg/m² iv on Days 1–5) and *cis*-dichlorodiammineplatinum(II) (DDP) (40 mg/m² iv on Days 1 and 4). The dose of DDP was diluted in 2 liters of 5% dextrose in ⅓ normal saline with 37.5 g of mannitol and 40 mg of Lasix and was given over 6 hours. Treatment B consisted of the same drugs used in treatment A plus procarbazine (75 mg/m² orally daily on Days 1–8) and vincristine (1.4 mg/m² iv on Day 1).

A mean of 4.1 courses (range, one to 12) in group A and 3.7 courses (range, one to 11) in group B was given to each patient.

RESULTS

In group A, there was one complete response lasting for 13 months in a patient with an unresectable soft tissue metastasis in the neck and there were five partial objective responses (> 50% regression) lasting from 2 to 9 months. In group B, two patients had partial objective responses of 3 and 4 months' duration respectively.

In summary, there were six objective responses among 16 patients in group A (42.5%) and two objective responses among 13 patients in group B (15.4%). Due to the small number of patients, the difference between the two treatment groups was not statistically significant.

In group A there was one severe (wbcs, < 1500/μl), one moderate (wbcs, 1500–2500/μl), and two mild (wbcs, 2500–3500/μl) instances of hematologic toxicity. There were also two patients with mild and one patient with marked high-frequency hearing loss. Five patients had decreases in creatinine clearance to < 65 ml/minute; three of these patients maintained normal BUN and serum creatinine levels, but one had rising BUN and creatinine levels while another developed acute renal failure from which he re-

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covered. In group B, there were two moderate and two mild instances of hematologic toxicity; one patient had marked and two patients had mild high-frequency hearing loss; four patients had decreases in creatinine clearance, but none had renal failure; and in two patients who developed paresthesias, vincristine was stopped.

There was no significant platelet toxicity in either of the two treatment groups.

Five patients in group A required dose modification, including two patients with significant nephrotoxicity in whom treatment was discontinued. Six patients in group B required dose modification because of toxicity.

DISCUSSION

In larger series, the response rate to DTIC alone, nitrosoureas, or combinations of DTIC with nitrosoureas and other drugs has been slightly < 20% (1), but in smaller series, response rates up to 30% are not uncommon (3–5). Numerous combinations have been listed (2,6). In some studies it was concluded that the addition of other agents to DTIC did not enhance antitumor activity (7). Other reports have suggested that chemoimmunotherapy may be more effective (8). Generally, efforts have been directed towards more effective combination chemotherapy (9).

In a previous report, DDP as a single agent was found to have some effectiveness as secondary chemotherapy for malignant melanoma (10). In the present study, DTIC with DDP or both of these drugs with procarbazine and vincristine were evaluated. The four-drug combination was slightly more toxic, although both regimens were generally well-tolerated. The two-drug combination of DTIC and DDP had a better response rate, but the difference was not statistically significant. DTIC and DDP definitely produced objective responses in this series and the combination probably deserves further study in a larger randomized series compared to DTIC alone.

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Treatment of Small Cell Bronchogenic Carcinoma With VM-26 [1]

Brief Reports and Preliminary Communications

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SUMMARY

VM-26 is active in small cell bronchogenic carcinoma even in patients resistant to previous chemotherapy. The overall response rate was 28%, with a response rate of 29% in patients resistant to previous chemotherapy. This response rate is similar to that obtained with other single agents including Adriamycin, vincristine, cyclophosphamide, and procarbazine. The mild subjective and objective toxic effects of the drug make it a potentially useful agent in the treatment of this tumor.

[Cancer Treat Rep 63:2011–2013, 1979]

VM-26 is a semisynthetic podophyllotoxin derivative which has activity in several human tumors including Hodgkin's and non-Hodgkin's lymphomas, bladder carcinomas, and brain tumors (1). Sklansky et al (2) have reported a response to VM-26 in a patient with intracranial metastatic small cell lung cancer, but Samson et al (3) noted no responses in 11 patients treated with this drug. The related compound, VP-16-213, is active in small cell lung cancer, leukemias, and testicular tumors (4,5). The cumulative response rate of small cell lung cancer to VP-16–213 is in the order of 25%–65% (4,6–9) and it is among the most active agents for this tumor type. In view of the structural similarity between VP-16–213 and VM-26, we have studied the efficacy of VM-26 in 25 patients with small cell lung cancer.

VM-26 has previously been administered iv daily for 5 consecutive days every 2–4 weeks in doses of 30 mg/m^2. Phase I toxicity data for this dose and schedule are scanty, but toxicity has usually been low. We initially used 100 mg/m^2 daily for 5 consecutive days, but thrombocytopenia was severe and the daily dose was subsequently reduced to 60 mg/m^2 for 5 days. The iv route was chosen because of the lack of information on the use of oral VM-26.

PATIENTS AND METHODS

All patients had histologically confirmed small cell lung carcinoma. Twenty-one had previously received combination chemotherapy with vincristine, cyclophosphamide, and Adriamycin (OCA); six patients had failed to respond, nine patients had relapsed during treatment, and six patients had relapsed after therapy had been discontinued, having received 500 mg/m^2 of Adriamycin. Four patients had received no prior treatment and were not eligible for the OCA protocol because of their poor general medical condition. Patients treated with the OCA protocol had also been randomized to receive central nervous system prophylaxis with either intrathecal methotrexate (ten patients) or systemic high-dose methotrexate (seven patients). Eighteen patients had also received radiotherapy (4000 rads) to the primary tumor site and to the mediastinum. Twenty-five patients (19 males and six females; 39–71 years old; median, 58 years) were treated. Five patients had disease clinically limited to one hemithorax and the ipsilateral supraclavicular fossa; the other patients had extensive disease. The performance status at the time of starting VM-26 (UICC criteria) included three patients with grade 0, eight patients with grade 1, five patients with grade 2, seven patients with grade 3, and two patients with grade 4. The interval between diagnosis and start of VM-26 was 0–70 weeks (median, 37 weeks). VM-26 was given by iv injection over 30 minutes in 250 ml of dextrose and saline daily for 5 consecutive days. The first five patients received 100 mg/m^2/day; the remaining 20 received 60 mg/m^2/day. Courses were repeated at intervals of 3 weeks. Patients with progressive disease after one course of therapy were taken off the study. Hemoglobin levels and differential, wbc, and platelet counts were recorded prior to each course of therapy and at 9 and 21 days following therapy. Twenty-channel biochemical profiles were measured before each course of treatment. The definition of complete response was the disappearance of all evidence of tumor. Partial response was defined as a > 50% reduction in the

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sum of the products of the maximum perpendicular tumor diameters, maintained for at least 4 weeks. Response duration was measured from the time of starting VM-26 treatment.

RESULTS

There were two complete responses and five partial responses giving a 28% response rate. Both complete responses occurred in patients with limited disease (performance status 2 and 3). Of the seven responders, four had had no previous radiotherapy and two had had no previous chemotherapy, while three patients had responded to OCA chemotherapy but had relapsed either during therapy (two patients) or after reaching the dose limit of Adriamycin and discontinuing chemotherapy (one patient). One of the five patients receiving 100 mg/m^2 of VM-26 responded (partial response) compared to six of the 20 patients receiving 60 mg/m^2 of VM-26. The duration of response from the time of starting VM-26 was 3–20+ weeks with a median of 9 weeks. One patient who was in good partial remission at 3 weeks and had early signs of tumor progression at 6 weeks is included as a partial response. There was no relationship between the site of the tumor and the response to chemotherapy, although none of the five patients with bony disease responded. Three of the six females and four of the 16 males responded. The performance status of responders was not significantly different from that of nonresponders (two with grade 1, two with grade 2, and three with grade 3). The intervals between diagnosis and the start of VM-26 were 0, 3, 9, 37, and 66 weeks for partial responders and 2 and 47 weeks for complete responders. Three patients had central nervous system metastases; two had had cranial radiotherapy prior to VM-26 and one had VM-26 alone. No response was seen in this latter patient.

Toxicity was not severe. Subjective tolerance was excellent and nausea and vomiting were not a problem. Significant leukopenia and thrombocytopenia (wbc count $<2.5 \times 10^9$/liter; platelet count $<75 \times 10^9$/liter) occurred in two patients 10 days after completion of chemotherapy with the high-dose schedule of VM-26, and in one of these patients the toxicity was severe (nadir wbc count 0.9×10^9/liter; nadir platelet count 40×10^9/liter). No patient receiving 60 mg/m^2 of VM-26 had myelosuppression by these criteria, although six patients had wbc counts of between 2.5 and 4.0×10^9/liter. It was not possible to assess the epilating effects of the drug since most patients were already alopecic following previous chemotherapy. There was no evidence of renal or hepatic dysfunction during VM-26 treatment. Two patients, both with known previous ischemic heart disease, developed congestive cardiac failure soon after starting therapy with VM-26. Neither of these patients had had previous radiotherapy but one had received Adriamycin. It is not clear whether these incidents were related to the administration of VM-26.

DISCUSSION

VM-26 in 5-day iv courses is active in the treatment of patients with small cell lung cancer. Seventeen patients in this study with tumors resistant to previous chemotherapy with OCA responded to VM-26, suggesting that this agent may be of value even in tumors resistant to standard chemotherapy. Tumor response was more frequent in women and in previously untreated patients, but the significance of these observations in this small number of patients remains to be determined. VM-26 has not previously been studied widely in small cell lung cancer. A previous report of VM-26 in small cell lung cancers noted no responses in 11 patients treated with VM-26 at 30 mg/m^2 on 5 consecutive days (3). Our study used at least double this dose and the 28% response rate suggests a dose-response relationship. However, there was no evidence of a higher response rate in our patients who received 100 mg/m^2 of VM-26.

Several reports indicate that VP-16-213 is an active drug in small cell bronchogenic carcinoma. Tucker et al (8) have used VP-16-213 given iv on 4 consecutive days in 47 patients with small cell lung cancer, 26 of whom had received no previous chemotherapy. Fifty-five percent of all patients and 50% of previously untreated patients responded. Other reports (6–10) have confirmed the activity of VP-16-213 in the treatment of small cell lung cancer. Our study shows that VM-26 is active in small cell bronchogenic carcinoma, and it is compatible with other reports that VP-16-213 and VM-26 have similar spectra of antitumor activity. Both drugs are effective in lymphomas and both may be of value in the treatment of nonseminomatous testicular tumors (1,5,10). In animals, both drugs show similar spectra of activity and are active in Ehrlich ascites tumor, sarcoma 180, sarcoma 37, Walker 256 carcinosarcoma, and L1210 leukemia.

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Phase I Evaluation of a Weekly Schedule of Anguidine [1,2]

Brief Reports and Preliminary Communications

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SUMMARY

A phase I evaluation of a weekly schedule of anguidine was undertaken as an alternative to the present continuous daily schedules. The dose ranged from 1.5 to 7.5 mg/m^2 given as an infusion over 3 hours. No myelosuppression was noted at any dose level. The toxic effects included nausea and vomiting, hypotension, CNS symptoms (confusion, hallucinations, and psychomotor seizures), chills, fever, and diarrhea. A dose of 5 mg/m^2 of anguidine produced acceptable toxicity.

[Cancer Treat Rep 63:2015–2017, 1979]

Anguidine (diacetoxyscirpenol) is the principal phytotoxic metabolic product of a plant fungus, *Fusarium equiseti* (1). Even though the molecular structure is well-known, its mode of action is uncertain. Anguidine appears to inhibit DNA and protein biosynthesis in HeLa cells with little or no effect on RNA synthesis. [4]

Anguidine was selected for phase I trials because of reproducible increases in lifespan in the P388 and L1210 murine leukemic models. Continuous therapy given ip on Days 1–9 in a range of 1.0–2.5 mg/kg/injection was found to be superior to intermittent therapy and was the only schedule that produced "cures." [4] Corbett et al [5] demonstrated that anguidine was effective in the treatment of a transplantable murine colon adenocarcinoma, in either a weekly or daily × 5 schedule. Anguidine was noted to be synergistic with 5-fluorouracil and cyclophosphamide. Phase I studies conducted at the M. D. Anderson Hospital and Tumor Institute (2) and the University of Kansas Medical Center (3) using a daily × 5 schedule reported that CNS symptoms (confusion and hallucinations) were the dose-limiting toxic effects. If the infusion was given over a 4- to 8-hour period, the CNS symptoms decreased, but more myelosuppression was seen. Goodwin et al (3) felt that a dose of 4.5 mg/m^2 given over a 4- to 8-hour period was the maximal dose of anguidine with no major side effects. The present study was undertaken to evaluate the maximum-tolerated dose and the toxicity of a weekly schedule of anguidine to be used alone and in combination with other agents (5-fluorouracil and cyclophosphamide). We wanted to test schedules applicable to outpatients and avoid hospitalization.

MATERIALS AND METHODS

Anguidine was supplied by the Division of Cancer Treatment of the National Cancer Institute (Bethesda, MD) in lyophilized form in 5-mg vials. Up to 5 mg was dissolved in 5 ml of sterile, nonbacteriostatic water. The clear, colorless solution was diluted in 250 ml of normal saline and given iv over 3 hours. The starting dose was 1.5 mg/m^2/week × 3–6 weeks iv. This dose was escalated to 3, 5, and 7.5 mg/m^2 if three or more patients were studied at the preceding level and no undue toxic effects were noted. For the most part, new patients were entered in the study at the maximum-tolerated dose utilized in preceding patients.

Patient eligibility criteria were (*a*) histologically proven diagnosis of a solid tumor; (*b*) previous chemotherapy, but not within the preceding 4 weeks; (*c*) normal hematologic parameters: granulocyte count > 2500/mm^3 and platelet count > 150,000/mm^3; (*d*) life expectancy of > 6 weeks and performance status > 50% (Karnofsky scale); and (*e*) signed informed consent in keeping with guidelines of the Department of Health, Education, and Welfare and the Institutional Human Investigations Committee.

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[2] Supported by Public Health Service grant CA-20255 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3] This study was performed by the Southeastern Cancer Study Group Committee on Gastrointestinal Malignancies. The following members participated: *P. A. DeSimone* (Veterans' Administration Medical Center, Lexington, KY), *F. A. Greco* (Vanderbilt University School of Medicine, Nashville, TN), and *H. F. Lessner* (University of Miami School of Medicine, FL).

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TABLE 1.—Weekly anguidine

Patient No.	Primary tumor	Dose (mg/m^2)	Total No. of doses	Hepatic involvement*	Toxicity
1	Rectal	1.5	5	–	None
2	Rectal	1.5	10	–	None
3	Colonic	1.5	3	–	None
4	Pancreatic	1.5	3	–	Mild nausea and vomiting
5	Renal	1.5	6	–	None
6	Adenocarcinoma of lung	1.5	7	–	None
7	Colonic	3.0	6	+	Mild nausea and vomiting
8	Pancreatic	3.0	3	–	Mild nausea and vomiting
9	Malignant melanoma	3.0	4	+	Nausea and vomiting
10	Malignant melanoma	3.0	3	–	Nausea and vomiting
11	Colonic	3.0	9	+	Mild nausea and vomiting, chills, and fever
12	Pancreatic	3.0	9	+	Mild nausea and vomiting, chills, and fever
13	Adenocarcinoma of lung	3.0	4	+	Severe nausea and vomiting, and fever
7	Colonic	5.0	9	+	Nausea and vomiting, chills, fever, and macular rash at the injection site
14	Colonic	5.0	10	–	Mild hypotension
15	Rectal	5.0	5	+	Nausea and vomiting, and fever
16	Adenocarcinoma of lung	5.0	5	–	Nausea and vomiting, chills, and fever
17	Small cell carcinoma of lung	5.0	3	+	Nausea and vomiting, chills, fever, rash, and mild hypotension
18	Malignant melanoma	5.0	5	+	Nausea and vomiting
19	Malignant melanoma	5.0	7	–	Nausea and vomiting, chills, fever, and mental changes
20	Malignant melanoma	5.0	4	–	None
21	Rectal	7.5	1	+	Severe hypotension, and fever
14	Colonic	7.5	2	–	Severe nausea and vomiting, hallucinations, and mild hypotension
22	Colonic	7.5	1	+	Nausea, vomiting, and diarrhea
23	Renal	7.5	4	–	Chills, fever, and psychomotor seizures

*Clinical or laboratory evidence of hepatic involvement by tumor including liver and spleen scan; – = no hepatic involvement present and + = hepatic involvement present.

Pretreatment evaluation included a complete medical history and physical examination, bone marrow aspirate and biopsy, performance status, cbc with platelet count, urinalysis, BUN, tumor measurements, SGOT, LDH, alkaline phosphatase, and total and direct serum bilirubin.

Twenty-nine patients were entered in the study but only 23 were evaluable for toxicity. Thirteen of these patients had a gastrointestinal malignancy (five colonic, five rectal, and three pancreatic). There were five patients with malignant melanoma, two with renal carcinoma, and one each with adenocarcinoma of the lungs, small cell carcinoma of the lung, and an unknown primary.

RESULTS

Table 1 shows patients by primary disease, presence of hepatic involvement, and toxicity. Two patients were treated twice, with each receiving one dose escalation. The doses ranged from 1.5 to 7.5 mg/m^2. Total granulocyte counts were always > 2500/mm^3 and total platelet counts were always > 150,000/mm^3. For the 1.5- and 3-mg/m^2 doses, the only toxic effect noted was mild nausea and vomiting. These symptoms lasted for 2–3 hours and started at the end of the infusion. As the dose was escalated to 5 and 7.5 mg/m^2, the nausea and vomiting became consistently more severe, lasting for longer periods of time (24–48 hours), and was not relieved by antiemetics. Patient 7 received 3- and 5-mg/m^2 doses but refused an escalation to 7.5 mg/m^2 because of the expected nausea and vomiting.

Febrile episodes, noted with 3-mg/m^2 doses, were usually low-grade (101°F orally), lasting 3–4 hours after infusion. Patient 17 experienced a fever of 101°F orally after the second course, and a fever of 103°F after the third course, accompanied by hypotension and a maculopapular rash. These symptoms cleared in 24 hours. At 5- and 7.5-mg/m^2 dose levels there were three episodes of mild hypotension (20–30-mm Hg drop in systolic pressure). Hypotension was not associated with any symptoms except lightheadedness or dizziness. Patient 14 experienced the same degree of hypotension at the 7.5-mg/m^2 dose level that was experienced at the 5-mg/m^2 dose level, while the degree of nausea and vomiting and CNS complications increased with the escalation. When given 7.5 mg/m^2 patient 21 had severe hypotension; blood pressure values were recorded as low as 60/40 1 hour following the infusion of anguidine. The patient was slightly confused and unable to walk. The hypotension was treated with iv fluids and slowly resolved over the next 18 hours. As the blood pressure returned to normal, the patient developed a fever of 102°F orally. No source of infection could be found. The fever subsided over the next 24 hours. At a dose of 7.5 mg/m^2, CNS symptoms appeared (psychomotor seizures, hallucinations, and confusion), lasting as long as 36 hours.

DISCUSSION

It has been demonstrated that 5 mg/m^2 of anguidine given iv in a weekly 3-hour infusion produces acceptable toxicity. This dosage is similar to that used at the University of Kansas Medical Center in a daily × 5 schedule. One of the goals of this study was to avoid hospitalization by using a weekly schedule. However, the risk of severe hypotension is significant enough that anguidine should be administered in a hospital setting.

Certain toxic effects (CNS and cardiovascular) have been noted to have an increased frequency when there was clinical and laboratory evidence of hepatic involvement by metastatic tumor (2,3). Eleven patients were treated at anguidine doses of 5 and 7.5 mg/m^2. Only two of the six patients with hepatic involvement had toxic effects, while three of the five patients without hepatic involvement had toxic effects.

Patient 6 had extravasation of anguidine in his skin on two occasions. No necrosis was noted, but a spotty erythematous rash was seen both in the area of extravasation and in areas far distant from the extravasation site. The erythema was accompanied by soft tissue swelling. Complete clearing was noted within 4 days.

This study was primarily an evaluation of the toxicity of weekly anguidine. It should be noted, however, that there were nine patients who received six or more injections of anguidine and none of these patients demonstrated regression of tumor size.

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Vindesine in the Treatment of Esophageal Carcinoma: A Phase II Study [1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Vindesine, a new vinca alkaloid, has shown therapeutic activity in several human malignancies. A phase II study in 26 patients with squamous cell carcinoma of the esophagus was performed. Sixty percent of these patients had received prior chemotherapy. The starting dose was 3.0 mg/m^2, which was escalated by 0.5 mg/m^2 to a maximum of 4.5 mg/m^2. Treatment was given once weekly for 7 weeks and every other week thereafter. Twenty-three patients were evaluable for response and toxicity. One complete remission (3 months), three partial remissions (5, 4, and 2+ months), and two minor responses (1.5 and 1 month) were seen. The major toxic effects were peripheral neuropathy, leukopenia, fever and myalgias, and alopecia. There was one drug-related death from leukopenia and sepsis. Vindesine has demonstrated therapeutic activity in esophageal carcinoma. Further studies with this agent are indicated.

[Cancer Treat Rep 63:2019–2021, 1979]

Vindesine (VDS) is a new vinca alkaloid with demonstrated activity in a variety of in vitro tumor systems (1). Phase I studies indicated that a dose of 3–4 mg/m^2 was an appropriate one for further trials (1). In phase II studies, VDS has caused objective responses in lung cancer (2), melanoma (3), and breast cancer (3). A phase II trial was performed at the Memorial Sloan-Kettering Cancer Center in patients with squamous cell carcinoma of the esophagus.

MATERIALS AND METHODS

Twenty-six patients with squamous cell carcinoma of the esophagus were entered in this study starting July 1977 and ending November 1978. In all cases, the diagnosis was confirmed by our Department of Pathology. Fifteen patients had received prior chemotherapy: 13 with *cis*-dichlorodiammineplatinum(II) (*cis*-platin) and bleomycin, one with bleomycin alone, and one with bleomycin and methotrexate. Twelve patients had had at least one surgical procedure: in five patients the primary lesion had been resected, either partially or completely, and in seven the esophageal lesion was unresectable. Twenty-four patients had received prior radiation therapy: 12 before or after surgery and 12 as the primary mode of treatment.

Patient characteristics are shown in table 1. Patients with a poor performance status were accepted in the protocol: eight patients had a Karnofsky performance status of ≤ 40.

VDS was given once weekly for seven doses and then biweekly until disease progression, or until toxicity (usually neurotoxicity) became severe enough to warrant its discontinuance. VDS was given as a rapid iv injection into the side arm of a running iv line. The starting dose was 3 mg/m^2. The dose was escalated by 0.5 mg/m^2/week to a maximum of 4.5 mg/m^2. An adequate trial was defined as at least 4 weeks of therapy or clear-cut disease progression after at least two doses.

TABLE 1.—Patient characteristics

Characteristic	
Total No. of patients—	
Entered	26
Evaluable	23
Males/females	14/12
Mean age in yrs (range)	58 (41–78)
Mean performance status (range)	60 (20–90)
No. of patients with—	
Prior treatment	
Surgery	12
Radiation therapy	24
Chemotherapy	15

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TABLE 2.—Previous therapy, site of metastatic disease, degree of response, and response duration among 6 objective responders

Patient No.	Prior therapy	Metastatic site	Response	Duration of response (mos)
1	Surgery, radiation therapy, *cis*-platin-bleomycin	Lung	CR	3
2	Surgery, radiation therapy	Lung, esophageal recurrence	PR	2+
3	Surgery	Liver	PR	5
4	Surgery, radiation therapy	Lung	PR	4
5	None	Lymph nodes, lung	MR	1.5
6	Radiation therapy, *cis*-platin-bleomycin	Subcutaneous	MR	1

A complete remission (CR) was defined as 100% regression of disease for at least 1 month. A partial remission (PR) was defined as at least 50% but < 100% decrease in the sum of the two longest perpendicular diameters of clearly measurable lesions (or, for liver metastases, a ≥ 30% reduction in the sum of the subcostal size of the liver measured at 5-cm intervals along both costal margins) for at least 1 month. For lesions in the esophagus, the barium esophagram had to show a ≥ 50% but < 100% reduction in tumor size. A minor response (MR) was defined as a definite decrease in tumor size but a < 50% decrease in the sum of the two longest perpendicular diameters. In all cases, no new lesions could appear during the remission period. If there was no change in the size of the lesions, the patient was considered a nonresponder. Informed consent was obtained from all patients prior to the initiation of therapy.

RESULTS

Twenty-three of the 26 patients entered in the study were evaluable for response and toxicity. Two of the three unevaluable patients died of disease within 2 weeks of initiation of therapy; one patient refused further therapy after one dose.

Among the 23 patients evaluable for response, there was one CR (3 months), three PRs (5, 4, and 2+ months), and two MRs (1.5 and 1 month) (table 2). CRs and PRs were associated with an improvement in or stabilization of performance status.

Responses were seen in patients both without prior chemotherapy (three PRs and one MR) and with prior chemotherapy (one CR and one MR). Responses were seen in pulmonary metastases (one CR and two PRs), hepatic metastases (one PR), and lymph node or subcutaneous metastases (two MRs). In all cases where disease was evaluable by esophagram, the patients had received extensive prior irradiation to the esophagus. One response in the esophagus was seen among nine patients who had had previous radiation therapy.

The median survival for all evaluable patients was 2.3 months from the start of VDS therapy. Of the six objective responders, one patient with a PR died at 5 months and one with an MR died at 1.5 months. Four patients are still alive, at 7+, 7+, 4+, and 2+ months.

The major toxic effects seen were peripheral neuropathy (50% of patients), leukopenia (defined as a wbc count of < 2000/mm^3) (8%), fever and myalgias (26%), and alopecia (40%). Severe constipation or ileus was not encountered. Mild constipation was treated with laxatives and stool softeners. Nausea and vomiting were not prominent complaints. There was one drug-related death, due to leukopenia and sepsis, in a heavily pretreated (chemotherapy and radiation therapy) patient. VDS was stopped because of severe peripheral neuropathy in one patient with an excellent PR; disease progression occurred soon after.

DISCUSSION

Currently, the major modalities of treatment for patients with squamous cell carcinoma of the esophagus are surgery and radiation therapy. The prognosis for patients with this disease remains dismal, with < 5%–10% of all patients surviving for 5 years (4). There is clearly a need for the development of new effective systemic agents, for use both in patients with still potentially curable disease as well as for palliation in those patients with advanced tumors.

The experience with single chemotherapeutic agents in esophageal carcinoma is rather scanty. Bleomycin, the most widely used single agent, has been reported to cause responses ranging from 0 to 60% (5–11). In a study of 14 patients with measurable metastatic disease, Ravry et al (10) saw no objective responses or symptomatic improvement. Kolarić et al (9) reported four responses in

15 patients receiving bleomycin alone; the median duration of response was 2.6 months. Overall, using the criteria of response as outlined in the current report, CR and PR are probably seen in 10%–20% of patients treated with bleomycin; the median duration of response is approximately 2 months. Methotrexate, methyl-GAG, and mitomycin C have been studied in small numbers of patients; occasional responses have been seen (5,12,13). Recently, we reported a 19% CR and PR response rate using the combination of *cis*-platin and bleomycin; an additional 44% of patients had MRs (14). The median duration of response in patients with metastatic disease was 6 months.

The current study indicates that VDS is capable of causing CR and PR in 17% of patients with an overall objective response rate of 26%. The duration of remission is at least as long as that reported in most bleomycin series. The majority of patients in this study had received extensive prior chemotherapy, and one third had a Karnofsky performance status of < 40. The induction of CR in a patient with pulmonary metastases, who had previously received six courses of *cis*-platin and bleomycin, is particularly encouraging. VDS is probably at least as active as bleomycin in esophageal carcinoma. Further studies with VDS as a single agent are indicated. In addition, the lack of cross toxicity, ease of administration, and different mode of action make VDS an attractive candidate for combination with *cis*-platin and bleomycin.

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Platinum Concentrations in Bile and Plasma Following Rapid and 6-Hour Infusions of *cis*-Dichlorodiammineplatinum(II) [1,2]

Brief Reports and Preliminary Communications

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cis-Dichlorodiammineplatinum(II) (DDP) is an antineoplastic agent with a broad spectrum of activity against human malignancies (1). Following iv injection of DDP, platinum is cleared from the plasma in a biphasic pattern, with a rapid initial half-life (< 1 hour) and a prolonged terminal half-life (2–5 days) (2,3). The percentage of administered platinum excreted in the urine differs widely among various reports in the literature (2–6), but, in all studies, urinary excretion does not account for the total platinum administered. Small amounts of platinum have been detected in bile in the dog following an iv dose of DDP (3); biliary excretion of platinum in man, however, has not been previously reported. In an attempt to modify the gastrointestinal and renal toxic effects of DDP, several investigators have given this drug by prolonged iv infusion (6–8). The present report examines the plasma, urine, and bile levels of platinum in a patient who received DDP as both a bolus and a 6-hour infusion.

MATERIALS AND METHODS

The patient studied was a 63-year-old male who had adenocarcinoma of the biliary tree with extensive hepatic metastases. Following a palliative resection with insertion of a permanent T-tube in July 1976, the patient was treated with a combination of cyclophosphamide, 5-fluorouracil, and cytosine arabinoside. The patient had stable disease for 2 years, but tumor progression occurred in September 1978. He was treated with 3 mg/kg (180 mg) of DDP given as a 15-minute iv infusion followed by a mannitol-induced diuresis (9). Symptomatic improvement was noted, and in October 1978 the patient was readmitted for another course of DDP. Laboratory values at that time included an alkaline phosphatase of > 1500 units/liter (normal range, 31–82), a bilirubin of 17.3 mg/dl (normal range, 0–1.0), and a creatinine clearance of 125 ml/minute. The patient was hydrated with 5% dextrose/0.45% NaCl at 150 ml/hour for 18 hours prior to treatment. DDP was given at a dose of 3 mg/kg as a 15-minute iv infusion followed by a mannitol-induced diuresis. Blood samples (7 ml) were drawn into heparinized tubes (Becton-Dickinson Vacutainer 3200KA) before treatment and at 5, 10, 15, and 30 minutes and 1, 2, 4, 24, and 48 hours after the end of the infusion. Bile was sampled from the T-tube every ½ hour for 4 hours, every hour for the next 20 hours, and four times daily for the next 2 days. Urine was collected at 24-hour periods for the first 2 days following treatment.

One month after treatment the patient was clinically stable. The alkaline phosphatase was 924 units/liter, the bilirubin was 12.2 mg/dl, and the serum creatinine was 0.8 mg/dl. Following hydration as previously described, DDP was given as a continuous iv infusion for 6 hours. Additional mannitol was not administered. Blood samples were drawn before treatment, at 30 minutes and every hour after the beginning of the infusion until its completion, and at 5, 15, and 30 minutes and 1, 2, 4, 24, and 48 hours after the end of the infusion. Bile was sampled every 2 hours for 48 hours, and urine was collected for 24 hours after the infusion was started.

Platinum was assayed by flameless atomic absorption spectrophotometry using a modification (10) of the method of Pera and Harder (11). Recovery of platinum added to pretreatment plasma, urine, and bile did not differ significantly from 100%.

RESULTS

The sequential concentrations of platinum in plasma and bile following rapid and 6-hour iv infusions of 3 mg/kg of DDP are shown in figure 1. The plasma clearance of platinum after the rapid infusion is biphasic, with a

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rapid distribution phase (half-life = 34 minutes) and a prolonged elimination phase (half-life = 107 hours). Following the 6-hour infusion, only an elimination phase is identified, with a half-life of 73 hours. Maximum plasma and bile platinum levels were higher after the 15-minute than after the 6-hour infusion. Following the 15-minute infusion, the maximum platinum concentration in the bile was reached at 1 hour, and platinum was not detectable in bile 5 hours later. Maximum levels were reached during the last 2 hours of the 6-hour infusion; platinum was not detectable in bile 4 hours after termination of the infusion. On each occasion, approximately 50 ml of bile was collected from the T-tube during the first 24 hours after beginning the DDP infusion. The total quantity of platinum excreted in bile could not be calculated because the biliary diversion was incomplete. The 24-hour urine excretion of platinum was 42% of the administered platinum for the rapid infusion and 35% for the 6-hour infusion.

DISCUSSION

While urinary excretion is the major route of platinum elimination following iv DDP, the administered platinum is not completely cleared by the kidneys. Employing ^{193}Pt DDP, Lange et al (4) demonstrated rapid urinary excretion of approximately 15%–30% of the administered platinum, but accumulation of platinum in the liver and kidneys was noted, with radioactivity in the bowel as well. Smith and Taylor (5) found no detectable radioactivity in fecal samples from one patient for 48 hours following injection. Abdominal scanning demonstrated a band of activity from below the liver toward the spleen in the region of the upper part of the intestine, but no activity was seen elsewhere in the bowel. Using flameless atomic absorption spectrophotometry, Litterst et al (3) confirmed the previously described accumulation of platinum in the liver and detected "only small amounts of platinum in the bile" of dogs given DDP.

This study demonstrates that platinum was excreted in the bile of a patient after DDP administration. The patient studied had considerable hyperbilirubinemia, and patients with normal biliary excretory function may excrete greater amounts of platinum in their bile. Concentrations of platinum in the bile reached 15% of the highest plasma level after the rapid infusion and 8% of the highest plasma level after the 6-hour infusion. Although fecal platinum concentrations were not measured, the presence of platinum in the bile is compatible with the findings on abdominal scanning following labeled DDP administration. If there is no enterohepatic circulation, biliary excretion may account for a small part of the platinum not excreted in the urine.

Moore et al (12) studied the distribution of ^{191}Pt administered iv in rats and found that platinum was excreted in nearly equal amounts in urine and feces. While not a major route of DDP elimination, biliary excretion may be important in the clearance of other platinum-containing antineoplastic drugs.

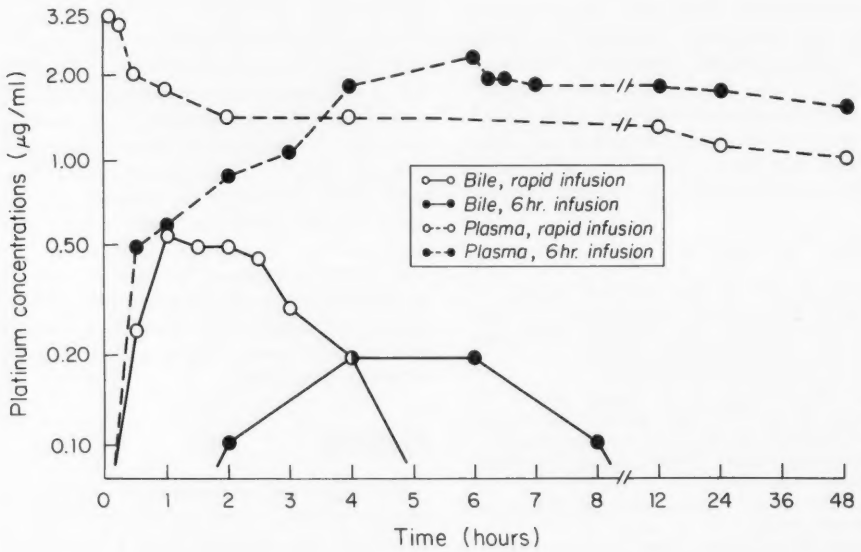

FIGURE 1.—Platinum concentrations in plasma and bile following DDP infusions.

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cis-Dichlorodiammineplatinum(II) in Advanced Soft Tissue and Bony Sarcomas: A Southwest Oncology Group Study [1,2]

Brief Reports and Preliminary Communications

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SUMMARY

***cis*-Dichlorodiammineplatinum(II) (DDP), at a dose of 15 mg/m²/day × 5 consecutive days, was administered to 68 evaluable patients with metastatic soft tissue and bony sarcomas. All patients, except one, had received extensive prior chemotherapy and had had progressive disease at the start of the study. Responses observed included one complete response in a patient with mesothelioma and three partial responses in patients with soft tissue sarcomas (7%). No responses were seen in 18 patients with bony sarcomas. Significant leukopenia and thrombocytopenia were observed in < 20% of evaluable courses, although two patients manifested life-threatening leukopenia (< 1000 cells/μl) and three had life-threatening thrombocytopenia (< 24,000 cells/μl). Nephrotoxicity was noted in < 25% of evaluable courses. Nausea and/or vomiting was recorded in 55% of evaluable courses. DDP is considered to be marginally active in the secondary treatment of metastatic sarcomas at this dose and schedule. Further studies of DDP in mesothelioma are indicated.**

[Cancer Treat Rep 63:2027–2029, 1979]

Clinical trials with *cis*-dichlorodiammineplatinum(II) (DDP) were initiated in 1971 under the auspices of the Division of Cancer Treatment of the National Cancer Institute (Bethesda, MD). Since then, numerous studies have confirmed antitumor activity in a variety of solid tumors including testicular, ovarian, head and neck, and bladder cancer (1–5). In April 1976, the Southwest Oncology Group (SWOG) began a study of DDP in advanced soft tissue and bony sarcomas. The results of that study are the basis of this report.

MATERIALS AND METHODS

Seventy-six patients from 21 institutions with histologically confirmed incurable soft tissue or bony sarcomas, who had failed to respond to higher priority treatment protocols, were entered in the study. Entrance requirements included: informed consent, measurable disease, an expected survival of at least 8 weeks, an absolute granulocyte count of ≥ 2000/μl and a platelet count of ≥ 100,000/μl, a BUN of ≤ 20 mg/100 ml, a serum creatinine of ≤ 1.7 mg/100 ml, and no evidence of obstructive uropathy. Prior chemotherapy, with recovery from the toxic effects of that therapy, must have been completed at least 3 weeks into the study.

DDP, supplied in 10-mg vials with 10 mg of mannitol and 9 mg of NaCl, was diluted in 10 ml of sterile water and given as a rapid iv injection. All patients received DDP at a dose of 15 mg/m²/day × 5 days and repeated at 28-day intervals, provided there was no evidence of impaired renal function and/or persistent myelosuppression at that time. One 5-day course of chemotherapy with at least a 14-day survival was considered sufficient to evaluate response with at least a 4-week survival necessary to evaluate toxicity. Patients were evaluated during the study with serial tumor measurements, BUN, serum creatinine, and other chemical and hematologic parameters.

Complete remission (CR) was defined as the disappearance of all clinical evidence of active tumor for a minimum of 4 weeks together with the disappearance of all tumor-related symptoms. Partial remission (PR) was defined as a ≥ 50% decrease in the sum of the products

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TABLE 1.—Histologic subtypes in evaluable patients treated with DDP

Histologic subtype	No. of evaluable patients
Soft tissue sarcomas	
Leiomyosarcoma	16
Synovial cell	5
Fibrosarcoma	4
Liposarcoma	4
Unclassified	2
Miscellaneous*	11
Bony sarcomas	
Osteosarcoma	16
Chondrosarcoma	1
Ewing's sarcoma	1
Other tumors	
Mesothelioma	6
Neuroblastoma	1

*Includes: lymphangiosarcoma, rhabdomyosarcoma, hemangiosarcoma, myxosarcoma, endometrial stromal, cystosarcoma phylloides, neurofibrosarcoma, fibroxanthoma, epithelioid, giant cell, and fibrous histiocytoma.

of the largest perpendicular diameters of all measured lesions lasting for ≥ 4 weeks in the absence of a significant increase in any one lesion or the appearance of new lesions. Disease progression was defined as a ≥ 25% increase in any measured lesion or the appearance of new lesions.

Seventy-six patients (39 males and 37 females) with a mean age of 45 years (range, 16–86 years) and a mean Karnofsky performance status of 69 (range, 40–90) were entered in this study. Eight patients (11%) were considered unevaluable for the following reasons: three patients died within 14 days of entry in the study and were considered early deaths, two patients were receiving other chemotherapy agents concurrently, two patients were lost to followup, and one patient received concurrent immunotherapy. Table 1 outlines the tumor types and the number of evaluable patients within each histologic subtype. Of the 75 patients who had received prior chemotherapy, 63 (84%) had had exposure to anthracyclines, generally as part of prior SWOG combination programs (table 2) (6–9).

RESULTS

Response

Four responses were noted in the study: one CR in a previously untreated patient with abdominal mesothelioma (5 months) and three PRs in patients with liposarcoma (8+ months), synovial cell sarcoma (6 months), and leiomyosarcoma (2 months). The three partial responders had received prior chemotherapy consisting of Adriamycin plus DTIC plus actinomycin D and cyclophosphamide plus vincristine plus Adriamycin plus DTIC. The overall response rate in soft tissue sarcomas was 7%. No responses were observed in 18 patients with bony sarcomas.

TABLE 2.—Previous chemotherapy with anthracyclines

Chemotherapy combination	No. of patients
Cyclophosphamide + vincristine + Adriamycin + DTIC	24
Cyclophosphamide + vincristine + Adriamycin + actinomycin D	15
Cyclophosphamide + DTIC + Adriamycin	8
Adriamycin	6
Adriamycin + DTIC	3
Adriamycin + DTIC + actinomycin D	3
Adriamycin + cyclophosphamide	2
Rubidazone + DTIC	2

Toxicity

Significant leukopenia (wbcs < 3000/μl) was observed in 16% of evaluable courses of therapy, while significant thrombocytopenia (platelets < 75,000/μl) was noted in 18% of evaluable therapy courses. There appeared to be no evidence of cumulative myelosuppression or any delay in administering subsequent courses of treatment. Two patients manifested life-threatening leukopenia (wbcs < 1000/μl) and three patients had life-threatening thrombocytopenia (platelets < 24,000/μl).

BUN elevation (>20 mg/100 ml) was noted in 24% of evaluable therapy courses while serum creatinine elevation (> 2.0 mg/100 ml) was noted in 5% of evaluable therapy courses. Nausea and/or vomiting was noted in 55% of evaluable courses. Ototoxicity was not observed.

DISCUSSION

This phase II study of DDP in patients with advanced soft tissue and bony sarcomas has demonstrated an overall response rate of 7% in the former and 0 in the latter. The 95% confidence limit of 1.5%–19.5% response rate suggests a low level of activity of DDP in this tumor category. In addition, in an ongoing broad phase II study at Wayne State University, no responses have been observed in eight patients with advanced soft tissue sarcomas receiving a higher dose of DDP (100–120 mg/m^2 every 3 weeks).[6]

Our finding of lack of activity of DDP in patients with advanced osteogenic sarcoma differs with the recent reports of Ochs et al (10) who noted one CR and four PRs among eight patients and Baum et al (11) who noted one

[6]Samson MK. Unpublished data, 1979.

CR among eight patients. Both of these studies involved patients in a pediatric age distribution, whereas the median age in our study was 30 years (range, 17–86 years). Additionally, in both the Ochs and Baum studies, the average dose of DDP administered per course was higher than that reported here. Other factors accounting for the differing response data are not readily apparent.

We conclude that, at the dose and schedule used in this study, DDP does not appear to have significant activity in advanced previously treated patients with soft tissue or bony sarcomas. However, since one CR was observed in a patient with mesothelioma, DDP should be further evaluated in this disease category.

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A Carcinoembryonic Antigen Standardization Experiment for the Conduct of Multi-Institutional Clinical Trials [1,2]

Brief Reports and Preliminary Communications

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(For the Gastrointestinal Tumor Study Group)

SUMMARY

The Gastrointestinal Tumor Study Group conducted a carcinoembryonic antigen standardization experiment to address the issue of reproducibility of assays performed at participating institutions and a central laboratory. Samples were drawn in quadruplicate from 37 patients, representing five participating institutions. Systematic biases were observed in four of the five institutions: two series were consistently higher and another two series were consistently lower than the corresponding central laboratory determinations. Significantly higher variations were found in three of the five series, comparing the sample variances associated with the matching assay series. Overall, the standard deviation associated with the central laboratory was one third that of the participating institutions. These results point toward the limitation of pooling multi-institutional carcinoembryonic antigen data. The use of multivariate models with parameters for institution comparison is proposed as a solution to this problem.

[Cancer Treat Rep 63:2031–2033, 1979]

The Gastrointestinal Tumor Study Group (GITSG) conducted a carcinoembryonic antigen (CEA) standardization experiment to examine the nature of statistical variation in results obtained by institutions and a central laboratory in the assaying of CEA plasma samples. Five institutions (Roswell Park Memorial Institute, University of Miami, University of California, University of Southern California, and Mt. Sinai Hospital-New York) participated in this study. Mallory Laboratory served as the central laboratory. Frontier Science and Technology Research Foundation coordinated the study.

MATERIALS AND METHODS

CEA plasma samples were to be drawn from ten patients within each participating institution. For each patient, an adequately large sample was drawn and divided into four equal aliquots, each capable of being assayed independently. Each aliquot was identified and labeled. Identification codes were sent to the GITSG Statistical Center. For each patient, two aliquots were sent to the central laboratory for processing according to usual laboratory procedure and the remaining two aliquots were processed within the institution laboratory. Explicit shipping instructions were given regarding packaging and delivery of aliquots to the central laboratory. Neither the central laboratory nor the institution laboratory had knowledge of the duplicate status of the specimens. All processed aliquots, together with the unique identification, were forwarded to the GITSG Statistical Center. Laboratories were instructed to use their standard assay procedures to replicate the clinical setting.

PURPOSE OF STUDY

The GITSG collects baseline and serial CEA data for all patients enrolled in group protocols. Both baseline and serial samples are routinely processed at the laboratories of participating institutions. Baseline CEA samples are drawn in triplicate, with two samples assayed at the participating institution and the remaining sample sent to Mallory Laboratory for assay.

The purpose of the study was to examine the variability of CEA assays within institutions and between partici-

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pating institutions and the central laboratory. At issue was whether CEA data from different institutions could be pooled in multi-institutional clinical trials.

RESULTS

Five institutions participated. Results were forwarded directly to the Statistical Center. Mallory Laboratory returned the results to the Statistical Center. A total of 37 patient samples were deemed evaluable for the purpose of this experiment. Thirteen remaining samples were declared unevaluable. Six samples were assayed via the direct method of assay (> 30 ng/ml), five samples were vaguely classified as being > 2.5 ng/ml, and the containers containing two samples were broken during shipping. In the remaining assays, the range of CEA averages was from 0.0 to 18.3 ng/ml, with each institution having at least three patients with CEA averages > 2.5 ng/ml.

A review of the institution and central laboratory results suggested that the variation in assay results was independent of the concentration of the CEA assays for the 37 samples analyzed. The central laboratory and two institutional laboratories assayed samples using the modified Hansen indirect Z-gel assay while the remaining three institutional laboratories conducted their assays using the Hoffmann-La Roche method.

Under the assumption of constant variation associated with each institution and the central laboratory, standard deviations were calculated. The results are presented in table 1. Central laboratory standard deviations were smaller than comparable standard deviations for four institutions. The results suggest that the standard deviation associated with the central laboratory was significantly less than that of the individual institutions.

Systematic bias was addressed by computing average CEA concentrations for each patient for the local institution and central laboratory. Assay averages for every patient for two institutions were systematically above those obtained by the central laboratory, and assay averages for every patient for two other institutions were systematically below those obtained by the central laboratory. This event is highly unlikely ($P < 0.001$). For each institution an L statistic was calculated to test whether institutional assays were significantly different from central laboratory assays. The t tests pointed to significant overestimation of CEA concentration by one particular institution. Overall, the patient averages were not significantly different between the pooled institutions and the central laboratory, suggesting that there were no consistent differences attributed to the shipping process.

Variation was addressed by computing the sum of squares of differences between the two assay results for each institution and the comparable central laboratory samples under study. Ratios of these sums of squares yielded F statistics under the hypothesis of equal variances. Differences between institutions and the central laboratory with respect to heteroscedasticity indicated a high degree of discordance on the parts of the institutions, with significantly higher variances in three of the five participating institutions. Summary test results are presented in table 2.

TABLE 1.—Institutional and central laboratory standard deviations

Institution No.	Standard deviation for—	
	Institution	Central laboratory
1	0.36 ng/ml	0.18 ng/ml
2	0.66 ng/ml	0.55 ng/ml
3	3.12 ng/ml*	0.78 ng/ml
4	0.36 ng/ml*	0.19 ng/ml
5	0.43 ng/ml*	0.66 ng/ml

*Hoffmann-La Roche assay method.

DISCUSSION

The high degree of variation associated with the systematic biases and variance has considerable impact on the analysis of those data collected from several different institutions. This experiment demonstrates that CEA data should not be immediately pooled from different institutions. An attempt to do so would result in additional error which would obscure the results of any endpoint analysis including CEA. From a statistical point of view, additional accrual would be required to reduce these additional sources of variation.

Initial effort should be directed at the standardization of assay methods and modification procedures. A standardization experiment should then be conducted to identify systematic bias and variation. Systematic bias may be addressed by adjusting empirical observations by the estimated institutional bias. Variation is more difficult to resolve. The appropriate method of analysis requires specific modeling techniques. It has been demonstrated (1) that an effective way of dealing with this situation is to include the institution effect in any statistical model for which each institution is compared to a baseline (or control) institution. This multivariate technique allows for the systematic biases and variance to be isolated through the corresponding institutional parameter. In

TABLE 2.—*P* values for differences and variances

Measure	Test	Institution				
		1	2	3	4	5
Difference	t test	NS*	NS	NS	NS	0.05
Variance	F test	NS	0.001	0.04	NS	0.03

*NS = not significant.

the multi-institution setting, for discrete endpoints (response or recurrence at a fixed time point), the logistic model (2) can be appropriate, while for the time-related endpoints (survival or time to recurrence), the Cox model (3) or exponential model (4) may apply. Statistical modeling can be of assistance in the presence of significant differences between institutions.

The major value of the central laboratory is to provide continuing quality control. If major institutional deviations can be identified, then either additional standardization experiments are in order or a review of assay procedures is warranted. The central laboratory must remain active during the duration of the study to ensure that no institutional differences develop. Assay procedures should be documented to promote uniformity in the multi-institutional setting. Without a central laboratory, sharply increased accrual objectives may be required to properly evaluate CEA data in the multi-institutional setting.

These results extend to other serologic tests and standardized assay procedures of possible prognostic value.

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Continuous Streptozotocin Infusion: A Phase I Study[1]

Brief Reports and Preliminary Communications

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SUMMARY

Streptozotocin (STZ) has shown antitumor activity against various tumors in man, but the clinical usefulness of this drug has been limited, mainly because of renal and gastrointestinal toxicity. Nineteen patients with advanced cancer of various types were given a mean dose of 3.4 g/m² of STZ by continuous iv infusion over 5–6 days each month for one or two monthly cycles. Basic serum and urine studies were performed immediately before and after each treatment cycle. Following STZ treatment, no significant changes in BUN or creatinine were seen. Four patients in whom initial tests for proteinuria were negative developed grade 1 or 2+ proteinuria after completion of the treatment cycle. No myelosuppression or renal failure was observed. Six patients had no nausea or vomiting, seven patients had nausea only, three patients had nausea and vomiting which were well-controlled with antiemetics, and three patients had uncontrollable nausea and vomiting. Confusion, lethargy, and depression were noted in five patients who had no prior central nervous system abnormalities; these effects appeared during treatment or in the immediate posttreatment period. Two patients with diffuse non-Hodgkin's lymphoma had complete remission, while several other patients had documented improvement. Although central nervous system toxicity may be a limiting factor, prolonged STZ infusions may have significant clinical promise.

[Cancer Treat Rep 63:2035–2037, 1979]

Antitumor activity from streptozotocin (STZ), a methylnitrosourea antibiotic, has been demonstrated in various tumors in man including lymphomas (1), islet cell carcinomas (1–7), carcinoid tumors (3,8), and various adenocarcinomas of the gastrointestinal tract.[4] The primary mode of action of STZ both in bacterial and mammalian cells is the inhibition of DNA synthesis, but it affects all stages of the cell cycle (4). The reconstituted drug is stable for at least 48 hours at 20°C between pH 3.8 and 4.7 under normal room-light exposure.[4] The mean biologic half-life of STZ is approximately 40 minutes following the administration of 1.5 g/m² by iv injection (9). STZ can rapidly penetrate tissues (9), and the metabolites of the nitrosourea portion of STZ enter the central nervous system (CNS) much more readily than those of BCNU or CCNU (10). The intensity and duration of antitumor therapy as well as the usefulness of this drug have been limited, mainly because of renal toxicity when the drug was given by rapid iv infusion (1–4,11). The renal toxicity has included proteinuria, glycosuria, hypophosphatemia, azotemia, and decrease in both serum bicarbonate and creatinine clearance. The drug has caused death secondary to renal failure in some cases (1,4,11).[4] At low doses STZ is essentially marrow-sparing, but hematologic toxicity has been reported, usually at high doses (1). Although there has been no report of overt clinical diabetes in non-insulin-producing tumors following STZ treatment in man, mild impairment of glucose tolerance has been reported (1,3).[4] Nausea and vomiting have been described as the most frequent side effects, occurring in from 75% to 98% of patients (1,3,4). Hepatotoxicity, as evidenced usually by elevations in SGOT and SGPT and rarely by elevations in alkaline phosphatase and bilirubin or by hypoalbuminemia, was found in from 15% to 67% of patients (1,4). Other miscellaneous side effects include fever (3), diarrhea (4), eosinophilia (3), pain at the injection site (2,3),[4] and one case of reduction in mental acuity for several days after STZ treatment (7).

The present report describes the initial experience with continuous STZ infusion in 19 patients with various advanced cancers.

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[4]Handelsman H, Broder LE, Slavik M, et al. Clinical brochure: streptozotozin, NSC-85998. Prepared by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, 1974, 68 pp.

MATERIALS AND METHODS

Patients

Nineteen patients with advanced metastatic cancer who had received extensive prior chemotherapy were treated. The treatment group consisted of eight patients with diffuse non-Hodgkin's lymphomas as well as patients with lung, breast, prostate, and gastrointestinal tumors. All patients underwent the following studies immediately before and after each STZ cycle: BUN, serum creatinine, urinalysis, total bilirubin, alkaline phosphatase, SGOT, SGPT, fasting serum glucose, hemoglobin, hematocrit, leukocyte count and differential, and platelet count. A few tests had to be omitted because of the inability to obtain urine or serum.

Drug Infusion

STZ was reconstituted in citrate buffer and administered as an infusion in 1 liter of normal saline, 5% dextrose in water, or 5% dextrose in water and 0.45% NaCl. The patients were given between 0.5 and 1 g/m^2/day of STZ for 5–6 days by continuous iv infusion each month for one or two cycles. The mean dose was 3.4 g/m^2/cycle. Five patients received two monthly cycles, 12 patients received one complete monthly cycle, and two patients received only 3 days of infusion. The pH of the STZ solutions used varied between 3.98 and 4.59 when tested by pH meter at several intervals over a 52-hour period (12).

RESULTS

Toxicity

None of the 19 patients had significant changes in BUN following STZ infusion, while 17 patients tested demonstrated no significant changes in serum creatinine. Of 11 patients with either no proteinuria or trace proteinuria before therapy, four had developed 1 or 2+ proteinuria by the completion of the treatment cycle. No patient experienced renal failure. All patients maintained a stable hemogram, although the majority of patients were anemic, neutropenic, or thrombocytopenic before therapy. One patient developed eosinophilia with a peak absolute eosinophil count of 4288/mm^3 for 1 week following the second cycle of treatment. Of 13 patients tested, seven had normal pretreatment and posttreatment fasting glucose levels, while six who previously either had abnormal fasting sugars or were receiving 5% dextrose in water infusions had mildly abnormal posttreatment values. Liver toxicity was difficult to evaluate, since 14 of 17 patients tested had pretreatment liver function abnormalities as revealed by one or more abnormal liver function tests, and ten patients had focal defects on liver scan suggesting tumor metastasis. Only one of three patients with initially normal liver function studies had a posttreatment abnormality, which was manifested by SGOT elevation from 20 to 58 IU/liter (normal range = 9–30 IU/liter). During each STZ cycle all patients were either afebrile or had no significant change in temperature, except for one neutropenic patient (leukocyte count, 1000/mm^3) whose temperature rose from 98°F to 103°F on the third day of therapy. Two patients developed local erythema and pain at the site of iv infiltration and/or infusion without tissue necrosis. Six patients had no nausea or vomiting, seven had nausea only, three had nausea and vomiting which were controlled with antiemetics, and three had intractable nausea and vomiting. Five patients who had no prior CNS abnormalities developed confusion, lethargy, or depression for several days during treatment or in the immediate postinfusion period.

Although 18 of the 19 patients in this study died of their disease between 10 days and 9 months following the initiation of STZ treatment, no patient deaths could be attributed to STZ toxicity.

Response

Several patients experienced subjective or objective improvement following STZ infusion. Two patients with non-Hodgkin's lymphoma experienced complete tumor regression for 6 months. Partial remissions in patients with non-Hodgkin's lymphoma included marked decrease (> 50%) in adenopathy (two patients), skin lesions (two patients), and hepatosplenomegaly (one patient). Six patients had remarkable pain relief following STZ treatment.

DISCUSSION

Although more patients and long-term followup data are needed before the efficacy of this method of STZ delivery can be fully evaluated, these studies suggest reduced renal, gastrointestinal, hepatic, and hematologic toxicity compared to previous reports. Proteinuria was the only evidence of renal dysfunction seen in our study. Evidence of renal damage as seen in the past, such as oliguria, azotemia, glycosuria, and elevation in serum creatinine, was not seen in our study, but delayed toxicity studies are needed in which STZ is given by continuous infusion. Nausea and vomiting were observed less often than in previous studies, which have reported an incidence of nausea and vomiting of up to 98% (4). Hematologic toxicity, which has been reported at high doses or after repeated courses (1,8), did not occur in our study. Eosinophilia, as seen in one patient, has been reported previously (1,3) and may suggest an allergic reaction to the drug. Hepatic toxicity with elevations of SGOT from 15% (1) to 50% (11) has been reported. All but three

patients in this study had abnormal pretreatment liver function studies due, in most cases, to tumor involvement, so it is difficult to compare our study with those of others. Only one patient with normal liver function showed a mild posttreatment elevation of SGOT. As in previous studies, frank diabetes was not seen in STZ treatment of non-islet cell tumors (3).[5] This report revealed the unexpected occurrence of CNS toxicity with continuous STZ infusions. Although the lethargy and depression experienced by some of our patients reversed within 2 or 3 weeks, this unexpected CNS toxicity may be a limiting factor for the use of prolonged STZ infusions. Although the CNS toxicity appeared at various dose ranges, we recommend that future trials begin at the lower dose range of 0.5–0.6 g/m^2/day for 5 days per monthly cycle until further toxicity data are available.

STZ given as a continuous infusion in this study resulted in minimal renal toxicity and impressive responses in patients with advanced non-Hodgkin's lymphoma. Further in-depth trials are warranted to better establish its full clinical potential.

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[5] *See* footnote 4.

Phase I Study of Iv Administration of Methanol Extraction Residue of Bacillus Calmette-Guérin [1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Four weekly iv doses, ranging from 10 to 640 μg/m², of the methanol extraction residue of bacillus Calmette-Guérin (MER) were given to 22 patients with advanced cancer in an effort to determine a maximum tolerated dose. Fever and chills for 24–48 hours were common at doses > 80 μg/m² but were prolonged for 3 days to 9 weeks at 640 μg/m². While moderate decreases in pulmonary vital capacity were seen at all dose levels, diffusing capacity decreased consistently only at higher doses. However, no roentgenographic changes suggestive of multiple lung granulomas were observed, such as were produced in dogs and mice by iv administration of MER. Autopsy failed to reveal granulomas in three patients. There were no objective tumor responses, nor were consistent changes observed in blood counts or skin tests to recall antigens. MER can be given iv with acceptable acute toxicity in doses up to 640 μg/m², at which point fever may be unduly prolonged. Patients given MER iv must be followed carefully for the development of interstitial pneumonitis.

[Cancer Treat Rep 63:2039–2041, 1979]

The methanol extraction residue (MER) is a crude extraction product of bacillus Calmette-Guérin (BCG). It is active alone and in combination with various therapeutic modalities in multiple animal tumor systems (1). When the drug is given as a single agent intradermally in man, objective regression of measurable lesions in advanced colon cancer has been reported (2). Iv administration is attractive because it avoids local skin toxicity and it might increase efficacy, since the MER is disseminated throughout the body.

In the dog, a fast iv push of doses from 2 to 20 mg/m² leads to cardiovascular collapse (3), but 20–30-minute infusions are free of acute toxicity. Multiple doses of 2 mg/m² in the dog and 15 mg/m² in the mouse are well-tolerated without detectable toxicity. In the dog there are no changes in arterial blood gases or liver function tests at this dose. At necropsy, however, granulomas were noted in the lungs and liver in both species (3).

MATERIAL AND METHODS

Eligibility for this study was restricted to patients with advanced cancer beyond the hope of cure and without reasonable hope of palliation with known therapeutic modalities. Each patient gave informed consent.

The starting dose was 10 μg/m² given weekly for four doses as a 30-minute infusion in 30 ml of normal saline, which was agitated at 5-minute intervals. Three patients were entered at each dose level, with each patient receiving four identical doses. Each successive dose level represented a doubling of the previous dose.

Biweekly serial studies included skin tests (streptokinase/streptodornase, mumps, trichophytin, *Candida*, and purified protein derivative [PPD]), posteroanterior and lateral chest roentgenograms, pulmonary vital capacity and single breath diffusing capacity, arterial blood gases, and cbc and SMA 18.

Twenty-two patients entered the study. The median age was 63 years, with a range of 34–78. Thirteen were female. The diagnosis was colon cancer in 11 patients, breast cancer in four patients, ovarian cancer in two patients, and melanoma, parotid cancer, renal carcinoma, gastric adenocarcinoma, and squamous cancer of the lung in one patient each.

Seventeen patients had undergone major surgery, six patients had received radiotherapy, and 21 patients had received chemotherapy. None of the patients had had prior immunotherapy with BCG or MER, but one had

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TABLE 1.—Fever as a function of MER dose

Dose level* ($\mu g/m^2$)	No of patients developing fever	Highest fever (°C)	Duration of fever (days)
10	0	—	—
20	1	39.8	2
40	0†	—	—
80	1	38.8	1
160	3	39.5, 40, 38.4	4, 2, 2
320	3	38.4, 40, 39.5	2, 1, 1
640	3‡	40, 39.4, 39.6	7, 3, 63

*3 patients were treated at each dose level.

†All 3 patients were febrile (38.2°C) at entry, but none had exacerbation of fever.

‡Excludes 1 patient treated once at 640 $\mu g/m^2$ without toxicity who died of disease 4 days later.

TABLE 2.—Mean parameter % change from pretreatment level (range in parentheses)

Parameter	Dose ($\mu g/m^2$)		
	10, 20, 40	80, 160	320, 640
Hemoglobin	−1(−23 to +16)	−15(−6 to +21)	−7(−14 to +15)
Granulocyte count	+34(−60 to +125)	−51(single value)	+15(−27 to +63)
Lymphocyte count	+89(−30 to +346)	−69(single value)	+14(−7 to +78)
Alkaline phosphatase	−5(−79 to +67)	+81(−21 to +248)	+303(−8 to +819)
Vital capacity	−9(−30 to +33)	−11(−25 to −3)	−11(−13 to −7)
Diffusing capacity	+8(−5 to +33)	−17(−22 to −11)	−20(−38 to −4)

been given sc *Corynebacterium parvum* and another had been given oral levamisole. Seventeen patients were ambulatory. Ten had metastases to the liver and six had metastases to the lung, five were febrile at entry, seven were anergic, and seven had PPD reactions ≥ 5 mm.

RESULTS

The median survival of the 22 patients entered in this study was 4 months, with a range of 4 days to 18 months. Nineteen patients survived the 6 weeks necessary to complete the protocol study, 13 patients survived ≥ 4 months, and six patients survived ≥ 6 months. Fever was the most consistent side effect observed at the higher doses (table 1). Two patients experienced mild nausea and vomiting. All patients experienced decreases in pulmonary vital capacity, perhaps related to progressive loss of muscle strength from advancing cancer. No consistent change in diffusing capacity was noted during the course of the study at 10, 20, or 40 $\mu g/m^2$. At 80 $\mu g/m^2$ and higher, consistent decreases in diffusing capacity were noted (table 2). In seven patients with pretreatment and posttreatment diffusing capacity measurements, there was a mean decrease of 19.2%. If four patients with known lung disease are excluded from the analysis, the mean decrease from the pretreatment value is 11.2%.

No changes were noted in arterial blood gases or chest films that could not be directly attributed to progression of the patient's underlying disease. No symptoms or physical signs attributable to MER-induced pulmonary disease were observed.

No consistent change in serum tests of liver function was noted at 10, 20, or 40 $\mu g/m^2$. At doses ≥ 80 $\mu g/m^2$, serum alkaline phosphatase rose ≥ 25% during the study period in nine of 12 patients. Six of these had known liver metastases, but the other three had 1.5- to 2.6-fold increases in alkaline phosphatase unaccompanied by known liver disease. One of these patients had a 3.5-fold increase in serum glutamic oxaloacetic transaminase as well.

No consistent change was noted in hemoglobin concentration, total wbc count, or the numbers of granulocytes, lymphocytes, eosinophils, or monocytes (table 2). No consistent changes were noted in delayed hypersensitivity to recall antigens. In only one patient was a negative tuberculin test converted to a positive one.

No antitumor efficacy was noted in the present study.

Ten patients had no change in their disease status and 12 patients had progressive disease during the 6 weeks of the study. Disease progressed in five of ten patients with liver metastases and in two of six patients with lung metastases.

Autopsy information is available for three patients, two who received four doses at 10 and 40 $\mu g/m^2$ each and one who received a single dose of 640 $\mu g/m^2$ and died of disease 4 days later. In no case were liver or lung granulomas seen. There was no evidence of any unusual lymphocytic activity around infiltrating tumor in any of these patients.

DISCUSSION

The results of the present study show that MER can be given iv to man at doses of $\leq$ 640 $\mu g/m^2$ as 30-minute infusions on a weekly schedule without prohibitive acute or subacute toxicity. Fever is universal after doses of $\geq$ 160 $\mu g/m^2$ and is prolonged for days or weeks after doses of 640 $\mu g/m^2$. Since the fever is associated with malaise and chills, a dose of 640 $\mu g/m^2$ is unlikely to be acceptable to patients for prolonged treatment periods. Whether the maximal clinically tolerated dose ($\sim$ 320 $\mu g/m^2$) is the dose capable of achieving optimal immune modulation in favor of the human host with cancer is unknown.

The high frequency and severity of fever found in the present study are in contrast with the results of Robinson et al where only 35%–50% of the patients developed fever (4). Perhaps more advanced disease or concomitant corticosteroid therapy contributed to a blunted febrile response in Robinson's patients.

Pulmonary diffusing capacity decreased in our study only at higher doses of iv MER, while vital capacity fell to a similar extent in all patient groups. A disproportionate decrease in diffusing capacity when compared to vital capacity suggests an interstitial process in the lung. This may reflect a granulomatous pneumonitis, such as that reported from intralesional MER (5–7), from iv MER (especially in patients who have received BCG therapy or have positive PPD skin tests [J. U. Gutterman, personal communication]), and from BCG given intradermally (8) or iv (9).

Evidence for MER-induced liver granulomas, as seen with iv BCG (9), is tenuous, hinging on three patients treated with higher MER doses whose alkaline phosphatase levels rose without other known liver disease.

In conclusion, MER can be given safely and tolerably as a slow iv infusion in doses up to 320 $\mu g/m^2$. Later trials should closely monitor pulmonary function tests and roentgenograms, as well as liver function tests, since iv MER may induce granulomas in these organs. An optimal dose of iv MER for immune modulation in the tumor-bearing host cannot be determined from this phase I study.

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Subsequent Cancer in Patients With Ewing's Sarcoma[1]

Brief Reports and Preliminary Communications

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SUMMARY

Among 31 long-term survivors of Ewing's sarcoma, two patients developed second primary cancers, compared to an expected number of 0.03 (relative risk = 72; 95% confidence limit = 8–259). One patient had renal medullary neuroblastoma, which is not known to be related to Ewing's tumor or its therapy. The second patient had a bone fibrosarcoma, arising at the primary tumor site, which was thought to be radiation-induced. The risk of radiation-induced bone sarcomas was lower, although not significantly so, than in a recently reported series of Ewing's tumor. These two reports suggest that patients with Ewing's sarcoma have a tendency to develop radiogenic sarcomas following primary megavoltage radiation therapy. The lowest radiation dose consistent with local tumor eradication should be employed to minimize the risk of subsequent radiogenic cancer.

[Cancer Treat Rep 63:2043–2046, 1979]

Improvements in the survival rates for a number of cancers following intensive chemotherapy and radiotherapy have raised questions about the late consequences of treatment, including carcinogenic potential. There is special concern with respect to childhood cancers, since young people may be particularly susceptible to carcinogenic exposures. A recent report of an excess risk of osteogenic sarcomas among long-term survivors of Ewing's sarcoma at the M.D. Anderson Hospital and Tumor Institute (MDAH) (Houston, TX) (1) prompted a review of our experience with this tumor.

METHODS

Between 1964 and 1978, 135 patients with Ewing's sarcoma were referred to the Radiation and Pediatric Oncology Branches of the National Cancer Institute (NCI), Bethesda, MD, for evaluation and therapy. An independent pathology review confirmed all histologic diagnoses. Patients were treated with 5000–6000 rads of megavoltage radiation to the primary tumor site, followed by varying combinations of chemotherapeutic agents as previously described (2). Many patients received 2000 rads of prophylactic brain irradiation (*see* table 1). Thirty-one patients who were disease-free 3 years after the date of initial therapy were considered at risk of a second primary cancer and formed the study cohort. None were lost to followup through December 1977. From these patients, the following data were abstracted: race, sex, age, year of diagnosis, type of therapy, site of primary tumor, and development of new primary cancers.

Expected numbers of cancers for all sites combined (excluding nonmelanoma skin cancer) and for specific sites were generated by applying the age-, race-, and sex-specific incidence rates from the Third National Cancer Survey (3) to the corresponding person-years at risk. Person-years were accumulated from the time of diagnosis of Ewing's sarcoma plus 3 years to the time of diagnosis of the second primary cancer, date of death, or closing date of the study (December 1977), whichever came first, using the USDR computer program (4). The person-year-rad (PY-rad) experience for the entire cohort was determined by multiplying each patient's person-years of observation by the radiation dose delivered

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TABLE 1.—NCI Pediatric Oncology Branch Ewing's sarcoma series: clinical data

		Ewing's sarcoma					
Patient No.	Race and sex*	Yr of diagnosis	Age at diagnosis	Site	Radiation (rads) to primary site	Chemotherapy‡	1978 status§
1	WM	1964	19	Femur	5636	S1	NED
2	WM	1965	20	Trunk	4580†	S1	Dead¶
3	WF	1968	13	Tibia	5000	S2	NED
4	WM	1969	8	Fibula	5000†	S2	A/M
5	WM	1969	12	Humerus	5000†	S3	NED
6	WF	1969	14	Mandible	5000	S2	NED
7	WF	1969	15	Femur	5000†	S2	NED
8	WF	1970	12	Tibia	5000†	S3	D/E
9	WF	1970	13	Femur	5000†	S3	NED
10	WF	1971	8	Fibula	4500	S3	D/E
11	WM	1971	10	Tibia	5000	Miscellaneous	NED
12	WM	1971	11	Mandible	5000†	S4	D/S
13	WM	1971	12	Trunk	5000†	S4	NED
14	WM	1971	13	Tibia	5000†	S3	NED
15	WM	1971	14	Femur	6400	S2	D/S
16	WM	1971	15	Fibula	5000†	S3	NED
17	WM	1971	18	Trunk	5000†	S3	NED
18	WF	1972	14	Trunk	4400†	S4	NED
19	WF	1972	15	Tibia	5000†	S4	NED
20	WM	1972	16	Tibia	5000†	S4	NED
21	WM	1972	17	Tibia	5000†	S4	NED
22	WF	1972	21	Foot	5000†	S4	NED
23	WF	1973	13	Femur	5000†	S4	NED
24	WM	1973	14	Femur	5000†	S4	NED
25	WM	1973	14	Trunk	0†	S4	NED
26	WM	1973	14	Tibia	5000†	S4	NED
27	WM	1973	15	Fibula	5000†	S4	NED
28	WM	1974	8	Tibia	5000	S4	NED
29	WM	1974	20	Femur	6000	S4	NED
30	WF	1974	24	Femur	6000	S4	NED
31	WF	1974	29	Foot	5000†	S4	NED

*WM = white male and WF = white female.

†Also received 2000 rads of prophylactic brain irradiation.

‡*See* ref 2 for details.

§NED = no evidence of disease (alive); A/M = alive with metastases; D/E = dead, Ewing's sarcoma; and D/S = dead, second primary cancer.

¶An iatrogenic death; NED at time of death.

to the primary site and summing. Radiation to other sites was not included in this calculation.

The strength of association was measured by the ratio of observed to expected cases and is referred to as the relative risk (RR). Exact 95% confidence limits (CL) were computed using Fisher's method. If the lower limit of the CL was > 1.0 (no association), the RR was considered significantly increased at the $P < 0.05$ level.

RESULTS

Table 1 presents the clinical data on the 31 white patients (19 males and 12 females) who qualified as long-term survivors. The demographic characteristics of this cohort resembled the overall series of 135 patients and other surveys of Ewing's sarcoma. All patients received radiotherapy to the primary tumor site (with one exception) and combination chemotherapy. Second cancers developed in two patients (patients 12 and 15), compared to an expected number of 0.03 (RR = 72; CL = 8-259). Calculations are presented in table 2, with the corresponding data from the MDAH series (1). Brief summaries of the two NCI patients follow.

Patient 1 (NIH No. 09–06–93–1)

An 11-year-old male developed Ewing's sarcoma of the mandible in 1971. The tumor was markedly PAS-positive. The patient received 5000 rads of irradiation to the primary tumor site, 2000 rads of prophylactic irradiation to the brain, and combination chemotherapy with intrathe-

TABLE 2.—Second primary cancers in patients with Ewing's sarcoma: comparison of NCI and MDAH experience

Variable	Series	
	NCI	MDAH*
Long-term survivors	31	23
Person-years of observation	112.6	112.0
Mean age (yrs) at diagnosis of Ewing's tumor	15.4	13.8
Mean survival (yrs)	6.6	?
Followup (yrs)		
Range	3–14	3–22
Median	6.1	5.3
Median radiation (rads) to primary site	5000	6000
PY-rads accumulated	558,530	555,556
Second cancers		
All sites		
Observed	2	3
Expected†	0.0279	0.0360
Observed/expected	72	83
95% CL	8–259	17–244
Bone		
Observed	1	3
Expected	0.0012	0.0012
Observed/expected	847	2400
95% CL	11–4715	500–8250
Mean age (yrs) at diagnosis of second cancer	17.2	21.7
Latency periods (yrs)‡	3.8/5.7	4.3/6.0/18.7§

*The index case in this series was omitted from the RR analysis to avoid ascertainment bias.

†Expected values based on age-race-sex-specific incidence rates from the Third National Cancer Survey.

‡Underlined data indicate radiation-related second primary.

§All radiation-related tumors occurred following megavoltage radiation *except* this one, which followed orthovoltage radiation.

cal methotrexate and systemic vincristine, Adriamycin, and cyclophosphamide. He did well until September 1975 when a right renal mass was noted. Detailed light and electron microscopic analysis of the nephrectomy specimen revealed a PAS-negative, catecholamine granule-containing tumor. The final diagnosis was renal medullary neuroblastoma. The patient developed disseminated neuroblastoma and died in December 1976.

Patient 2 (NIH No. 08–84–19–4)

Ewing's sarcoma of the femur was diagnosed in this 14-year-old male in 1971. Therapy included 6400 rads of irradiation to the primary tumor, followed by combination chemotherapy with vincristine and cyclophosphamide. A pathologic fracture developed at the tumor site, requiring surgical fixation. He remained well and free of disease until January 1976, when a mass recurred at the site of the initial tumor. Biopsy revealed a fibrosarcoma arising in the femur. This tumor soon became widely disseminated, and the patient died in October 1976.

DISCUSSION

Until recently, the prognosis for patients with Ewing's sarcoma was so dismal that few patients survived to experience long-term complications of therapy. With the improved survival following combined radiation and chemotherapy, an increasing number of patients are now at risk of late effects (2,5). A review of published reports of second neoplasms following Ewing's sarcoma revealed nine patients, eight of whom had solid tumors (including six with bone cancers) arising within irradiated areas (1). To this experience, Strong et al added four patients with osteogenic sarcoma developing at the primary tumor site (1); a patient with acute myelomonocytic leukemia was also reported (6). To date, all of these 14 patients with second primary cancers have received radiotherapy, with 11 also being given chemotherapy.

The four cases of osteogenic sarcoma following Ewing's tumor at the MDAH were thought to be radiogenic in origin, yet those patients who received adjuvant chemotherapy were at significantly greater risk than those

treated with radiotherapy alone. Among the MDAH patients, the cumulative actuarial 10-year risk of second primary cancers was 35% (± 15%). As shown in table 2, the NCI and MDAH series are similar in size, person-years of observation, PY-rads of observation, and expected numbers of second cancers. However, the MDAH cohort had several very long-term survivors and generally received more radiation (median dose per patient: 6000 versus 5000 rads), while the NCI patients tended to receive more chemotherapy.

The second primary cancers in the NCI series consisted of (*a*) a renal neuroblastoma, a rare tumor arising outside the radiation port and not previously related to Ewing's sarcoma or its therapy, and (*b*) a bone fibrosarcoma arising within the radiation port at the original site of Ewing's tumor. Although the significance of renal neuroblastoma in one patient is uncertain, it is well-documented that bone fibrosarcoma can be induced by ionizing radiation (7). It is unclear whether the pathologic fracture in this case, and in two other reports (1,8), contributed to the radiation-induced sarcomas. Local recurrence of Ewing's sarcoma following high-dose, megavoltage radiotherapy typically occurs within 2 years of treatment, and thus is an unlikely explanation for the cancers observed at the original primary site (9).

We observed one radiogenic cancer during 558,530 PY-rads of observation for an estimated 1.8 radiation-related cancers per million PY-rads, four times less than that reported by Strong et al (1). This difference (one case per 558,530 PY-rads versus four cases per 555,556 PY-rads) was not statistically significant ($P = 0.19$ exact). Although the risk of radiogenic cancer in our series was the same as that reported by Li et al (10) in their survey of second malignancies in children, these estimates are not comparable since Li et al included radiation delivered to other than the primary tumor site in their calculations. If the prophylactic brain irradiation received by most NCI patients is included in the calculations (an approach we do not recommend), the radiogenic cancer risk becomes 1.4 cases per million PY-rads. This issue is moot for the MDAH series since only one patient received radiation beyond the primary tumor site. In that study, the higher risk estimate (7.2 cases per million PY-rads) suggests that the MDAH experience may be atypical for reasons not yet apparent. Furthermore, if there were a synergistic effect between radiation and chemotherapy, as suggested by Strong et al (1), one might have expected to see a greater risk among the NCI patients who tended to be more aggressively treated with combination chemotherapy.

Variations in followup and radiation dose may have contributed to the different results in the two studies, since the MDAH patients included several very long-term survivors and typically received 1000 rads more than NCI patients. The single radiogenic tumor in the NCI series occurred in one of the very few patients who received > 6000 rads to his primary tumor. Accurate dose-response data for human bone cancers induced by high doses of external ionizing radiation are not available. A recent report has suggested a linear dose-response curve, and a low risk of radiation-induced sarcoma in the dose range used in modern radiotherapy (11). The limited animal data based on particle irradiation also suggest a linear relationship between radiation dose and risk of developing bone sarcomas (12).

In summary, it appears that patients with Ewing's sarcoma who receive large doses of megavoltage radiation are prone to new bone sarcomas in the irradiated area. There are no data to suggest that patients with Ewing's tumor have either an intrinsic predisposition to second cancers (which might be unmasked by improved survival) or a special sensitivity to the carcinogenic effects of ionizing radiation. Since 5000 rads of primary irradiation appear sufficient to achieve local control of Ewing's sarcoma (9), particularly when used in conjunction with adjuvant chemotherapy, it may be advisable to use this lower dose routinely in hopes of reducing the risk of second primary cancers. Since the median latent period for radiogenic bone cancers (7,10) exceeds the average duration of followup in both studies, it is likely that these reports understate the true risk, which should be clarified by surveys of larger groups of patients followed for longer periods of time.

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Phase II Trial of Pyrazofurin in Advanced Head and Neck Cancer [1,2]

Brief Reports and Preliminary Communications

Edgar Cheng, Violante Currie, and Robert E. Wittes [3,*]

Although several antitumor agents possess activity against advanced head and neck cancer (1,2), complete remission is rarely achieved, toxicity is often impressive, and duration of remissions is generally brief. Obviously, agents with a greater therapeutic index are urgently needed.

Pyrazofurin (PZF), a c-ribonucleoside antibiotic isolated from the broth filtrates of *Streptomyces candidus*, blocks de novo pyrimidine biosynthesis by inhibiting orotidylate decarboxylase. In the initial clinical trials with this drug (3,4), responses were noted in patients with breast cancer, mycosis fungoides, and acute myelogenous leukemia. The established toxic effects were mucositis, nausea and vomiting, myelosuppression, and occasional rash (5–7). At the Memorial Sloan-Kettering Cancer Center, disease-oriented phase II trials of PZF in advanced melanoma (5), lung carcinoma (6), colorectal carcinoma (7), and soft tissue sarcoma (8) showed no evidence of useful activity. The present study was designed to evaluate the potential utility of this drug in advanced head and neck cancer.

MATERIALS AND METHODS

Between September 1976 and October 1977, 22 patients with histologically proven epidermoid head and neck cancer were entered in the trial. All patients had complete physical examination, 12-channel screening profile, cbc including platelet count, chest roentgenogram, and scintiscan when indicated.

Therapy was started at a dose of 4–5 mg/kg given by rapid iv injection after solution in normal saline. Doses of drug were repeated at weekly intervals until either dose-limiting toxicity or response was achieved. When such toxicity was attained, appropriate reduction in dose was employed in subsequent treatments which were continued until disease progression. When no toxicity was achieved, doses were escalated in most patients by 1 mg/kg at weekly intervals to a maximum of 7.0 mg/kg. Of the 22 patients receiving treatment, all were evaluable except for one who was lost to followup after the second dose.

Partial response (PR) was defined as a decrease of at least 50% of all measurable disease for at least 1 month. Measurable disease was defined as the sum of the two greatest perpendicular diameters. Minor response (MR) was defined as an objective tumor decrease which was less than that required for a PR.

As shown in table 1, the median initial performance status was 70. More than 90% of the patients had had prior radiation therapy and the majority had had prior surgery and chemotherapy. Most patients had disease

TABLE 1.—Patient characteristics (22 patients)

Characteristic	
Median age in yrs (range)	56 (20–80)
Sex	
Males	13
Females	9
Median initial performance status* (range)	70 (50–90)
No. of patients	
Prior therapy	
Radiation therapy only	3
Surgery + radiation therapy	4
Surgery + chemotherapy	1
Radiation therapy + chemotherapy	8
Surgery + radiation therapy + chemotherapy	6
Distribution of primary disease sites (epidermoid)	
Oropharynx	9
Nasopharynx	3
Oral cavity	5
Larynx	2
Lower lip	1
Ear	1
Unknown primary	1
Site of measurable disease	
Primary site	13
Neck	14
Bone	1
Lung	2

*Karnofsky scale.

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TABLE 2.—Toxic effects from PZF therapy

	No. of patients
No. of PZF courses	
2	3
3	6
5	13
Toxic effect	
Mucositis	18
Wbc count nadir (cells × 10^3/mm^3)	
3.0–3.9	1
2.0–2.9	1
< 2.0	0
Hemoglobin decrease (g/100 ml)	
0–1.9	2
2.0–2.9	6
3.0–3.9	4
≥ 4.0	7
Platelet count nadir (cells/mm^3)	
50,000–100,000	2
< 50,000	0
Response to therapy	
PR	1
MR	1
Progression	19

recurrence at the primary site and/or in the neck at the time of treatment.

RESULTS

As shown in table 2, most of the patients had three or more doses of PZF. The major dose-limiting toxic effect was mucositis; anemia was also common, as had been previously reported (6). Leukopenia and thrombocytopenia were mild and generally of no clinical consequence. One patient with epidermoid carcinoma of the right pharyngeal wall, who had a performance status of 90 and had received prior radiation therapy and chemotherapy, experienced a PR lasting 5 months; another patient with epidermoid carcinoma of the oropharynx, who had a performance status of 80 and had received prior radiation therapy only, experienced an MR lasting 4 months.

DISCUSSION

Twenty-two patients with advanced epidermoid carcinoma of the head and neck region were entered in a phase II trial of PZF. In general, the treatment is well-tolerated. Mucositis is the most frequent side effect encountered but is manageable with an appropriate reduction in dose. Of the 21 evaluable patients, there were one PR and one MR (response rate, 4.8%; 95% confidence limits, 0.12%–23.8%). Although the majority of the patients had already failed surgery or radiation therapy or other chemotherapy, the activity of PZF is still disappointing, especially when compared with that of other agents given to similar patients (1,2,9). Although nine patients had received only two or three courses of therapy, their diseases progressed in the presence of mucositis or myelosuppression or both. Therefore, we conclude that PZF in this dose and schedule has no therapeutic value in patients with advanced epidermoid carcinoma of the head and neck.

In animal solid tumor systems (10), PZF caused up to 90% prolongation of life in rats with Walker 256 carcinosarcoma ascites tumor. Mammary carcinoma 755, Gardner lymphosarcoma, and X5563 plasma cell myeloma were inhibited > 50% by PZF. However, the S91 melanoma, sarcoma 180 ascites tumor, and Ehrlich ascites solid tumor showed no significant biologic responses (10). It is not surprising then to observe the variability in responses to PZF in clinical trials. Perhaps with a different dose schedule or with the application of uridine rescue (11), the therapeutic effectiveness of PZF could be improved.

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Treatment of Metastatic Pancreatic and Gastric Adenocarcinomas With 5-Fluorouracil, Adriamycin, and Mitomycin C (FAM)[1]

Brief Reports and Preliminary Communications

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SUMMARY

Twenty-six patients with metastatic pancreatic or gastric adenocarcinoma were treated with 5-fluorouracil, Adriamycin, and mitomycin C. One complete and five partial responses were observed among 15 patients with pancreatic adenocarcinoma (40% response rate) and six partial responses were observed among 11 patients with gastric adenocarcinoma (55% response rate). Responders had a significantly longer survival than nonresponders in both groups.

[Cancer Treat Rep 63:2049–2051, 1979]

Pancreatic and gastric adenocarcinomas are the fourth and fifth causes of cancer-related deaths in the US (1). While the incidence of gastric adenocarcinoma is decreasing, the incidence of pancreatic adenocarcinoma is on the rise. Pancreatic adenocarcinoma presents a particular dilemma to the clinician because it is frequently difficult to diagnose, and, once the disease is diagnosed, treatment is rarely curative. Similarly, although the diagnosis of gastric adenocarcinoma is more easily made, the surgeon frequently finds disease that is beyond the scope of curative resection at the time of laparotomy. While the combined-modality therapy may offer long-term disease control in patients with regional gastric adenocarcinoma, patients with regional pancreatic adenocarcinoma still die from this disease despite radiation therapy and 5-fluorouracil (5-FU) (2). Patients with metastatic gastric or pancreatic adenocarcinoma usually die from their disease within 2–3 months, and the use of the fluorinated pyrimidines has not substantially altered the natural history of these diseases. The treatment of these two neoplasms with Adriamycin has produced beneficial responses in 20%–30% of patients, and has led to the development of a three-drug program of 5-FU, Adriamycin, and mitomycin C (FAM) for the treatment of gastric adenocarcinoma as reported by Macdonald et al (3) and Haller et al (4). We have employed a similar regimen for the treatment of metastatic pancreatic and gastric adenocarcinomas since 1977 and herein report our results.

PATIENTS AND METHODS

From July 1, 1977, to December 31, 1978, 26 consecutive untreated patients with metastatic pancreatic adenocarcinoma (15 patients: six males and nine females; average age, 57.5 years [range, 34–83]) or gastric adenocarcinoma (11 patients: seven males and four females; average age, 54.8 years [range, 31–67]) were treated at the Michael Reese Hospital and Medical Center. All patients had metastatic disease as defined by a careful physical examination, a chest roentgenogram, liver-spleen and bone scans, and an abdominal ultrasonogram. All patients had histologic or cytologic proof of disseminated pancreatic or gastric adenocarcinoma. Performance status was initially determined by the Eastern Cooperative Oncology Group criteria. Sites of metastases are shown in table 1.

All patients received the FAM regimen, slightly modified from that reported by Macdonald et al (3), as follows: 5-FU, 500 mg/m^2 on Days 1, 8, 21, and 28; Adriamycin, 30 mg/m^2 on Days 1 and 21; and mitomycin C, 10 mg/m^2 on Day 1. Cycles of FAM were repeated every 42 days and weekly cbcs were performed in all patients to monitor hematologic toxicity. The following dose modifications were employed dependent upon the wbc and platelet counts: wbcs > 4000/mm^3 and platelets > 100,000/mm^3 = 100% dose; wbcs > 3000/mm^3 and platelets > 100,000/mm^3 = 75% dose; wbcs > 2000/mm^3 and platelets > 75,000/mm^3 = 50% dose; and wbcs < 2000/mm^3 and platelets < 50,000/mm^3 = no chemotherapy.

Responses were determined by the following criteria. Complete response (CR) was defined as the complete

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TABLE 1.—Patient characteristics, response to therapy, and survival

Patient No.	Age	Sex	Performance status*	Metastatic sites	Response	Change in weight (%)	Survival (mos)	Status
				Pancreatic adenocarcinoma				
1	67	M	0	Liver	CR	0	17	Alive
2	52	M	2	Liver, peritoneum, skin	PR	–3	13	Alive
3	52	M	3	Liver	PR	0	6	Alive
4	62	F	3	Liver, pleura	PR	+15	13	Alive
5	60	F	1	Liver, ascites	PR	0	15	Alive
6	61	F	1	Liver, lung	PR	0	19	Alive
7	45	F	2	Liver, abdominal masses	NR	–17	3	Dead
8	75	F	1	Skin	NR	–20	4	Dead
9	67	F	2	Liver, skin	NR	–10	4	Dead
10	79	F	3	Liver	NR	–15	4	Dead
11	68	F	1	Liver, peritoneum	NR	–25	4	Dead
12	73	F	1	Liver	NR	–10	3	Dead
13	41	M	0	Liver, bone	NR	–10	4	Dead
14	83	M	2	Liver	NR	–15	2	Dead
15	34	M	0	Liver, skin	NR	–10	2	Dead
				Gastric adenocarcinoma				
16	61	F	1	Ascites, peritoneum	PR	0	17	Dead
17	64	M	0	Abdominal masses	PR	–5	19	Alive
18	65	M	0	Abdominal mass, ascites	PR	–5	7	Alive
19	59	M	0	Liver, abdominal mass	PR	–2	4	Alive
20	67	F	3	Liver, abdominal mass	PR	+12	10	Dead
21	36	M	0	Liver	PR	+5	4	Alive
22	64	M	0	Liver	NR	–10	2	Dead
23	37	F	1	Liver	NR	–15	2	Dead
24	65	M	2	Liver	NR	–16	7	Dead
25	53	F	3	Liver	NR	–17	1	Dead
26	31	M	0	Liver, ascites	NR	–10	7	Dead

*Eastern Cooperative Oncology Group scale.

disappearance of all tumor as determined by physical examination and radiographic and scintigraphic studies and partial response (PR) was defined as a 50% reduction of measurable lesions as determined by physical examination and radiographic and scintigraphic studies. In the case of hepatomegaly, a 30% reduction in the overall liver span and accompanying improvement on liver scan were required. In the evaluation of malignant ascites, the complete disappearance of fluid as detected by physical examination was required. No response (NR) was defined as stable or progressive disease.

Survival was calculated from Day 1 of chemotherapy, and no patient was excluded from this analysis. Survival curves were compared by the Gehan modification of the generalized Wilcoxon test (5).

RESULTS

The results of treatment are shown in table 1. There were one CR and five PRs among 15 patients with pancreatic adenocarcinoma (40% response rate) and six PRs among 11 patients with gastric adenocarcinoma (55% response rate). The median survival for all patients with pancreatic adenocarcinoma was 3.7 months, with a projected median that will be > 13 months for patients with CR or PR (fig 1). Patients with disseminated pancreatic adenocarcinoma who had an NR with FAM had a median survival of 2.8 months. Patients who had a CR or PR with FAM had a significantly longer survival ($P = 0.001$).

The median survival of patients with metastatic gastric adenocarcinoma treated with FAM was 6.7 months, with a projected median survival of 16.5 months for responders (CR + PR) and 2 months for nonresponders (NR) ($P < 0.01 > 0.005$).

Patients who responded to the FAM regimen had either no change or a slight decrease in body weight (3%–5%). In two instances, weight gain was associated with an increased sense of well-being by patients receiving FAM; a loss of > 5% of body weight was an ominous prognostic sign. A low initial performance status did not preclude response to FAM since three of five patients with a performance status of 3 responded to treatment. There was only one treatment-related death in the entire series of 26 patients (patient 7) due to *Candida* sepsis. There were only two febrile episodes secondary to *Can-*

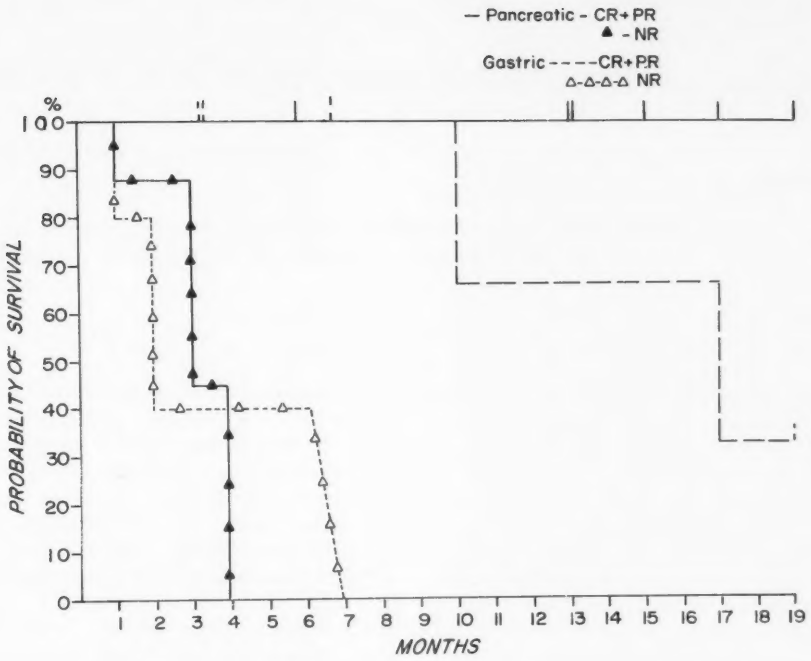

FIGURE 1.—Survival curves of 15 patients with pancreatic adenocarcinoma and 11 patients with gastric adenocarcinoma treated with FAM. The 6 patients with pancreatic adenocarcinoma who responded to FAM have a median survival of > 13 mos; nonresponders have a median survival of 2.8 mos ($P = 0.001$). Projected median survival for responders with gastric adenocarcinoma to FAM is 16.5 mos, and median survival for nonresponders is 2.0 mos ($P > 0.01 < 0.005$). Upright lines refer to patients still alive; gastric (Δ) and pancreatic (▲).

dida and *Escherichia coli* sepsis in 100 treatment cycles. Hematologic toxicity was chiefly manifested by myelosuppression occurring 14–19 days after FAM was initiated and consisted of granulocytopenia (median, 1150 cells/mm^3/patient/cycle). Thrombocytopenia (median, 114,000 cells/mm^3/patient/cycle) was frequently observed on Day 35.

DISCUSSION

Disseminated pancreatic and gastric adenocarcinomas are ultimately fatal neoplasms. The encouraging reports by Macdonald et al (3) and Haller et al (4) on the use of FAM led us to modify this regimen and employ it for the treatment of disseminated pancreatic and gastric adenocarcinomas. Our results indicate a beneficial response to FAM in pancreatic adenocarcinoma with responders enjoying a long survival (median of > 13 months). Additionally, our experience with this regimen in gastric adenocarcinoma has also shown similarly long survivals in responders (median of 16.5 months). These results must be interpreted cautiously, since randomized trials have yet to be performed with this regimen.

It is our belief that the FAM regimen should be used as therapy for pancreatic and gastric adenocarcinomas in randomized trials and as an adjunct to surgery or radiotherapy for the treatment of less-advanced disease.

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Phase II Study of Hexamethylmelamine Alone and in Combination With Mitomycin C and Vincristine in Advanced Breast Carcinoma [1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Fifty-three patients with metastatic breast carcinoma were randomized to treatment with hexamethylmelamine (HMM) as a single agent versus a three-drug regimen of HMM, vincristine, and mitomycin C (HOM). All patients had received prior treatment with 5-fluorouracil, Adriamycin, and cyclophosphamide with or without methotrexate. HMM alone was used in a dose of 300 mg/m²/day × 14 days every 21 days. In the HOM regimen, the HMM dose was 200 mg/m²/day × 21 days, the vincristine dose was 1.5 mg on Days 1, 8, and 15, and the mitomycin C dose was 12 mg/m² once every 6 weeks. No objective responses were observed with HMM in 15 evaluable patients. The HOM regimen resulted in five partial responses among the 27 evaluable patients. Gastrointestinal toxicity was the limiting toxicity of HMM, and thrombocytopenia was the major toxicity of the HOM regimen.

[Cancer Treat Rep 63:2053–2056, 1979]

Hexamethylmelamine (HMM) is a new synthetic antitumor drug with alkylating agent properties (1). It has been studied extensively, and in broad phase II studies including many tumor types, HMM showed a cumulative response rate of 25% (14 of 56 patients) in metastatic breast carcinoma (1–3). More recently, Lichinitser et al, in a phase II study of HMM in advanced breast cancer, reported objective responses in 21 of 37 patients (56%) (4). All of these trials were carried out in patients with minimal or no prior chemotherapy. Because of reports indicating a lack of cross-resistance between HMM and alkylating agents (5), we believed that a phase II study of HMM used as a single agent in metastatic breast cancer patients who had received prior treatment with standard drugs was justified. In addition, we attempted to utilize HMM in combination with mitomycin C and vincristine because of the reported activity of these two agents in breast cancer (6,7). The results of this study are described in this report.

PATIENTS AND METHODS

From October 1977 through July 1978, 53 patients with a biopsy-proven diagnosis of breast cancer were entered in this study. All patients had evidence of progressive disease following prior chemotherapy with a combination regimen of 5-fluorouracil (5-FU), Adriamycin, and cyclophosphamide or cyclophosphamide, methotrexate, and 5-FU. Patient characteristics are summarized in table 1. All patients had measurable or evaluable metastatic sites and a performance score (Eastern Cooperative Oncology Group) of ≥ 3. Patients with significant impairment of liver or renal function (serum bilirubin level > 1.5 mg/dl and serum creatinine level > 1.5 mg/dl respectively) were not eligible for the study. Patients were required to have an absolute granulocyte count of > 1500/mm³ and a platelet count > 100,000/mm³ before chemotherapy was initiated. Written informed consent was obtained from each patient before chemotherapy.

Pretreatment evaluation included complete blood cell counts and serum chemistry determinations including SMA 100 and carcinoembryonic antigen. Staging work-up included chest roentgenogram, a complete bone sur-

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TABLE 1.—Patient characteristics

Characteristic	Treatment regimen	
	HOM	HMM
No. of patients entered	30	23
Median age in yrs (range)	47 (36–74)	57 (41–72)
No. of patients with—		
Prior treatment*		
Endocrine therapy	25 (83%)	18 (78%)
FAC-CMF	25 (83%)	18 (78%)
FAC only	5 (17%)	5 (22%)
Dominant disease sites		
Visceral	16 (53%)	14 (61%)
Bone	11 (37%)	7 (30%)
Soft tissue	3 (10%)	2 (9%)
No. of organ sites involved		
1	15 (50%)	9 (39%)
2	4 (13%)	6 (26%)
3	6 (20%)	6 (26%)
≥ 4	5 (17%)	2 (9%)

*FAC = 5-FU, Adriamycin, and cyclophosphamide. CMF = cyclophosphamide, methotrexate, and 5-FU.

vey, bone and liver scans, and photographs of cutaneous lesions.

Patients were randomly allocated to treatment with HMM alone or the combination regimen of HMM, vincristine, and mitomycin C (HOM). The dose of HMM used as a single agent was 300 mg/m²/day × 14 days, repeated at 21-day intervals. The total dose was split into three doses and taken after meals and at bedtime. The dose schedule for HOM was as follows: HMM, 200 mg/m²/day × 14 days every 3 weeks; vincristine, 1.5 mg on Days 1 and 8 every 3 weeks; and mitomycin C, 12 mg/m² on Day 1 every 6 weeks. After the initial experience with HOM, the overlapping myelotoxic effects of HMM and mitomycin C led us to change the HMM schedule to Days 1–21 every 6 weeks and the vincristine schedule to Days 1, 8, and 15 of each 6-week cycle of therapy. Wbc and platelet counts were performed once weekly and prior to each course of treatment. The doses of HMM and mitomycin C were decreased by 25% if the previous course of treatment caused excessive myelosuppression, defined as a nadir granulocyte count of < 1000/mm³, a nadir platelet count of < 75,000/mm³, or both. Vincristine was withheld if neurotoxicity led to excessive muscle weakness that interfered with the patient's ability to function at nearly normal levels. Excessive gastrointestinal toxicity required a 25%–50% reduction in HMM dose or its discontinuation if toxicity was intolerable. HMM was temporarily withheld if platelet counts dropped to < 75,000/mm³ or if the total granulocyte count decreased to < 1000/mm³.

The criteria for response to chemotherapy were standard. Partial response (PR) was defined as a ≥ 50% decrease in the size of measurable lesions without evidence of new lesions. Recalcification of lytic bone metastases was also recorded as PR. Minor remission (MR) was defined as a > 25% decrease in tumor size. Patients with no appreciable change in evaluable disease accompanied by symptomatic improvement were classified as stable. An adequate trial was defined as 6 weeks of treatment with full doses of all drugs. An increase in the size of preexisting lesions by ≥ 25% or the development of new lesions constituted progressive disease.

RESULTS

Fifty-three patients were entered in the study; 30 received HOM and 23 received HMM. On the HOM arm, three patients were not evaluable, one because he died on Day 11 of the first course of treatment and one because of a major protocol violation. The third patient was lost to followup. In the HMM arm, eight patients were not evaluable for the following reasons: early death, three patients; inadequate trial because of intolerance to the drug, three patients; and lost to followup, two patients. The remaining 42 patients were evaluable for response: 27 in the HOM arm and 15 in the HMM arm. The HMM arm of the study was closed early because of lack of antitumor activity and similar reports from other institutions.

Response

In the HOM group, five patients achieved a PR, three patients achieved an MR, and eight patients had stable disease, while 11 patients showed progression of disease. PRs were observed in patients with lesions of the liver (two), pleural effusion (two), bone (one), nodes (one), and chest wall (one). The duration of response measured from initiation of chemotherapy was similar in all categories of response, with a median of 5 months and a range of 3–14+ months. The median number of HOM courses given was two (range, one to eight). In the HMM arm, there were no PRs and one MR; three patients had stable disease and 11 had progressive disease. The duration of tumor control in the four responsive patients was 4, 5, 6, and 7 months. The patient who achieved MR had regression of the chest wall nodules.

Toxicity

Forty-eight patients were evaluable for toxicity: 27 patients in the HOM group and 21 patients in the HMM group. The incidence of various toxic effects is shown in table 2 and the severity of hematologic effects is shown in table 3. Gastrointestinal side effects such as anorexia,

TABLE 2.—Nonhematologic toxicity

Toxic effect	No. of patients (%) with toxicity*	
	HOM	HMM
Anorexia and nausea	27 (100)	21 (100)
Vomiting	18 (67)	8 (38)
Neurotoxicity	14 (52)	2 (10)
Nephrotoxicity	2 (7)	—

*Patients with inadequate data on toxicity are excluded.

nausea, vomiting, and weight loss were the most frequent toxic manifestations of both treatment regimens. HMM was primarily responsible for the gastrointestinal toxicity, and the symptoms generally persisted during the time the patients were taking this medication. Nine patients (19%) required reduction in the HMM dose and six (12%) eventually refused to take it beyond the first 3 weeks of therapy. Most patients who received more than two courses of HOM had paresthesias, and vincristine had to be discontinued in seven patients (26%) because of significant peripheral neuropathy. Patients receiving HMM alone had no evidence of peripheral neuropathy, although two patients developed neurologic symptoms possibly secondary to HMM. One patient experienced extreme nervousness, insomnia, and twitching of leg muscles after completing five courses of therapy and refused further treatment. Another patient experienced significant generalized weakness that could not be attributed to other causes. Both patients were treated with pyridoxine without benefit. Mild impairment of renal function, probably secondary to mitomycin C, was observed in two patients receiving HOM. Both had normal renal function before receiving this chemotherapy but gradually developed a twofold rise in serum BUN and creatinine levels accompanied by proteinuria. Renal dysfunction persisted after HOM was discontinued.

Myelosuppressive toxicity is summarized in table 3. Thrombocytopenia was the dose-limiting toxic effect of the HOM regimen and it was cumulative with repeated courses of treatment. The median nadir platelet count was 77,000/mm^3 with the first course of HOM and 57,000/mm^3 with the second course. Two patients experienced thrombocytopenia (< 20,000 cells/mm^3) that required platelet transfusions. Myelosuppression from HMM was minimal. Five patients had no decrease in blood cell counts at all. Only five patients had thrombocytopenia with platelet counts < 100,000/mm^3; four of them had extensive bone marrow replacement with the tumor.

COMMENTS

Despite previous reports suggesting activity of HMM in breast carcinoma, we found no response to HMM in 15 breast cancer patients refractory to first-line chemotherapy. Similarly, Denefrio and Vogel (8) have recently reported a lack of activity of HMM in previously treated patients with breast cancer. In this regard, HMM is not unlike many other drugs, which, although active in untreated breast cancer, have shown a significant decrease or loss of activity when used in patients with prior exposure to cytotoxic agents. Thus, HMM, despite modest activity in untreated breast cancer patients, offers no advantage over many other drugs with similar activity and accordingly would not be useful in this disease.

The response rate achieved with HOM (19%) in this study is in the same range as that expected from mitomycin C used as a single agent. It is doubtful that HMM contributed to the response observed with HOM. Vincristine, which has a response rate of approximately 20% in untreated patients, may or may not have added to the antitumor activity of HOM. Neuropathy from vincristine did not permit its use beyond 6–8 weeks. Although HMM also causes neurotoxicity, our patients did not appear to have more neurotoxicity than that expected from vincristine itself.

Although the first attempt with chemotherapy in metastatic breast cancer patients results in response rates of 60%–75%, the majority of responders develop progressive disease after 12–18 months of achieving a response. No cytotoxic regimen has produced response rates > 20% in patients who have become resistant to the

TABLE 3.—Hematologic toxicity

	Treatment regimen	
	HOM	HMM
Median lowest wbc count (cells × 10^3/mm^3) (range)	2.9 (0.8–4.9)	3.9 (2.2–7.0)
Median lowest absolute granulocyte count (cells × 10^3/mm^3) (range)	1.4 (0.1–2.8)	1.8 (1.0–4.5)
Median Day (range)	21 (8–42)	21 (14–21)
Median lowest platelet count (cells × 10^3/mm^3) (range)	77 (18–266)	150 (26–300)
Median Day (range)	28 (8–42)	21 (14–31)

four most active agents, ie, 5-FU, cyclophosphamide, methotrexate, and Adriamycin. Mitomycin C is one of the few drugs with potential usefulness after patients are no longer responsive to these four drugs. However, the short duration of the responses achieved with mitomycin C and the severe myelotoxicity which permanently impairs the bone marrow reserve compromise its usefulness as a single agent. Furthermore, the myelotoxicity of mitomycin C interferes with its successful utilization in combination with other antitumor drugs.

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Alterations of Lactate Dehydrogenase Isoenzymes With Subacute Adriamycin Toxicity [1,2]

Brief Reports and Preliminary Communications

Harry M. Olson* and Craig F. Shannon [3,4]

SUMMARY

Single doses of Adriamycin (ADR) and isoproterenol in CDF rats produce significant increases in total serum lactate dehydrogenase (LDH) activity, LDH isoenzymes, and LDH–1/LDH–2 ratio. Isoproterenol produced significant increases in LDH–1, LDH–2, and LDH–3 and the LDH–1/LDH–2 ratio; ADR produced significant increases in all isoenzymes, reflecting widespread toxicity, with peak levels of total LDH, LDH isoenzymes, and LDH–1/LDH–2 ratio at 48 hours after injection. While the increased LDH–1/LDH–2 ratio may suggest myocardial leakage with ADR administration, the results are too nonspecific to conclude that the heart is the primary source of serum LDH–1 and LDH–2.

[Cancer Treat Rep 63:2057–2059, 1979]

In a previous investigation (1), a significant increase in total serum lactate dehydrogenase (LDH) activity was reported with both subacute single and divided high doses of Adriamycin (ADR) in rats. Serum LDH activity may arise from several tissue sources. However, isoenzyme distribution (zymogram) in rat sera may allow a determination of the tissue source(s) of increased serum activity (2,3). In the rat, studies have shown high endogenous levels of LDH isoenzymes 1 and 2 in myocardium (2,3).

Our original hypothesis (1) was that ADR produces a direct effect on the myocardial cell membrane that permits leakage of LDH isoenzymes 1 and 2 into the blood. The objectives of this study were to determine total serum LDH and isoenzyme patterns in the rat following single-dose ADR administration. The specificity of these results would then be compared to LDH isoenzyme activity following administration of the known cardiotoxic agent isoproterenol (ISO) (4) in the rat.

MATERIALS AND METHODS

Female CDF rats, 34–40 days old, with a weight range of 38–44 g, were injected ip with ADR at a single dose of 20 mg/kg (bulk ADR kindly provided by Drs. R. L. Wolgemuth and J. Luce, Adria Laboratories, Inc, Columbus, OH). DL-ISO HCl (Sigma Chemical Corp, St. Louis, MO) was injected at a dose of 85 mg/kg ip (50–55-day-old rats), a dose known to produce myocardial necrosis in rats (4). ADR was prepared at a concentration of 2 mg/ml and ISO was prepared at a concentration of 20 mg/ml in sterile isotonic (0.85%) saline immediately before injection. Control animals received an equivalent volume of saline ip.

Rats were killed in groups of up to ten each at 12, 24, 36, 48, and 60 hours after ADR. Rats receiving ISO were killed at 6, 12, 24, and 36 hours. Control rats were killed at the same time points as treated animals. A terminal serum sample was obtained from the abdominal aorta under ether anesthesia and the blood was immediately centrifuged at 2500 rpm for 10 minutes. All serum samples were assayed for total LDH and electrophoresed within 4 hours after collection. An aliquot of serum was assayed in duplicate for total LDH activity with a Cary Model 15M spectrophotometer (340 nm). Lactate (lithium salt) was used as substrate at a final concentration of 35 nM at 30°C, pH 8.6. Polyacrylamide gel electrophoresis was performed according to the method of Dietz and Lubrano (5).

LDH and isoenzyme activity is reported as milliunits per milliliter of serum. Isoenzyme concentration was determined by calculation as a percent of total LDH activity (estimate of area beneath the curve) multiplied by the total LDH activity of the sample. Statistical evaluation was performed using Student's t test (6).

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TABLE 1.—Total serum LDH and LDH isoenzyme activity after 20 mg/kg of ADR

LDH isoenzyme	Serum LDH and LDH isoenzyme (milliunits/ml)*					
		Time (hrs)				
	Control	12	24	36	48	60
LDH-1	4.8 ± 0.6	5.6 ± 1.2	7.0 ± 1.0	14.2 ± 2.8†	25.2 ± 5.4†	20.3 ± 3.3†
LDH-2	9.7 ± 1.2	20.8 ± 1.5†	23.0 ± 2.4†	25.8 ± 5.1†	38.9 ± 9.4†	32.2 ± 7.2†
LDH-3	5.1 ± 0.7	8.4 ± 1.2†	11.2 ± 1.4†	18.1 ± 3.9†	27.2 ± 4.4†	19.1 ± 3.9†
LDH-4	3.3 ± 0.8	5.9 ± 0.9	5.0 ± 0.5	13.4 ± 4.0†	18.5 ± 2.3†	14.2 ± 3.9†
LDH-5	9.9 ± 2.0	22.2 ± 4.5†	19.0 ± 2.7†	17.0 ± 3.4	24.3 ± 4.4†	25.0 ± 8.2
Total LDH	33.1 ± 2.8	59.3† ± 5.7	64.1† ± 4.2	85.9† ± 12.6	120.2† ± 19.6	110.7† ± 26.3

*Mean ± SD.
†$P < 0.05$, or greater, compared to control.

TABLE 2.—Total serum LDH and LDH isoenzyme activity after 85 mg/kg of ISO

LDH isoenzyme	Serum LDH and LDH isoenzyme (milliunits/ml)*				
		Time (hrs)			
	Control	6	12	24	36
LDH-1	6.0 ± 1.5	53.4 ± 12.7†	16.1 ± 0.8†	10.1 ± 0.4	8.6 ± 0.6
LDH-2	9.6 ± 0.6	68.8 ± 17.1†	17.0 ± 0.8†	7.4 ± 0.8	10.4 ± 1.1
LDH-3	3.4 ± 0.1	22.1 ± 5.8†	5.2 ± 0.4†	4.5 ± 1.6	5.2 ± 1.5
LDH-4	0.7 ± 0.4	10.2 ± 8.6	1.1 ± 0.2	1.8 ± 0.6	0.9 ± 0.5
LDH-5	4.8 ± 0.5	25.2 ± 15.0	5.4 ± 0.8	7.2 ± 1.6	2.1 ± 0.6
Total LDH	24.4 ± 1.2	179.7† ± 59.2	44.8† ± 2.5	31.1 ± 5.0	27.2 ± 3.7

*Mean ± SD.
†$P < 0.05$, or greater, compared to control.

TABLE 3.—Ratio of LDH-1/LDH-2 for ADR and ISO

Drug (dose)	LDH-1/LDH-2 ratio						
		Time (hrs)					
	Control	6	12	24	36	48	60
ADR (20 mg/kg)	0.45 ± 0.02	–	0.26* ± 0.05	0.30* ± 0.03	0.56* ± 0.03	0.66* ± 0.02	0.66* ± 0.06
ISO (85 mg/kg)	0.47 ± 0.01	0.78* ± 0.01	0.95* ± 0.02	1.38* ± 0.11	0.83* ± 0.03	–	–

*$P < 0.05$, or greater, compared to control.

RESULTS AND DISCUSSION

Mean total serum LDH activity increased following ADR injection in rats (table 1). Enzyme activity after 20 mg/kg was increased significantly compared to controls as soon as 12 hours, and remained significantly increased at all time points evaluated. Evaluation of isoenzyme activity revealed significant increases in all LDH isoenzymes at some time points compared to controls (table 1), reflecting the widespread toxic effects of this agent. At 12 hours, a more than twofold increase in LDH–2 was observed and was sustained at 24 hours. By 36 hours, LDH–1 was increased and both isoenzymes reached peak mean activity at 48 hours. LDH–3 was significantly elevated at all time points. LDH–4, as LDH–1, was not significantly increased until 36 hours and achieved maximum mean activity at 48 hours. LDH–5 activity was elevated significantly at 12, 24, and 48 hours after ADR injection. Except for LDH–5, all isoenzymes achieved maximum mean activity at 48 hours after injection.

Total serum LDH increased rapidly after ISO injection with peak enzyme activity at 6 hours and sustained elevation at 12 hours, compared to control rats (table 2). Isoenzyme activity showed significant increases only in LDH–1, LDH–2, and LDH–3 at 6 and 12 hours after injection, corresponding to the significant elevation of total LDH, and reflecting rapid and selective myocardial necrosis (4). An explanation for the increase in LDH–3 is uncertain, although it may originate from endothelial cell damage (7).

The ratio of LDH–1 to LDH–2 in rats receiving ADR and ISO is shown in table 3. An initial decrease in the LDH–1/LDH–2 ratio compared to controls was noted with ADR at 12 and 24 hours, with a subsequent increase at, and following, 36 hours. At 48 hours, when peak total and isoenzyme levels occur, the ratio is highest at 0.66. ISO produced an immediate increase in the LDH–1/LDH–2 ratio at 6 hours, with a continuous increase to a maximum of 1.38 at 24 hours.

The results of this study show significant increases in total serum LDH activity, LDH isoenzymes, and LDH–1/LDH–2 ratios following single doses of ADR and ISO. ISO produces selective increases in LDH isoenzymes 1, 2, and 3, with an increase in the LDH–1/LDH–2 ratio. Isoenzymes LDH–1 and LDH–2 occur in greatest proportion in myocytes, and are released following cardiac necrosis (8). Furthermore, an increased LDH–1/LDH–2 ratio has been used as an indicator of acute myocardial damage in man (9). ADR, by virtue of producing cytotoxic damage to numerous tissues, including gastrointestinal and myeloid tissue, causes increases in all isoenzymes at some time points. In addition to the heart, the only other organ known to contain significant amounts of LDH–1 and LDH–2 is the kidney (10). However, based on our data, the widespread toxicity produced by ADR in the rat at this dose precludes stating that the specific source of LDH–1 and LDH–2 is myocardial. Further investigations are planned to determine whether LDH isoenzyme evaluation will be useful as a marker of anthracycline cardiotoxicity in the rat.

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Phase II Evaluation of Maytansine in Patients With Advanced Head and Neck Cancer [1,2]

Brief Reports and Preliminary Communications

E. T. Creagan,* T. R. Fleming, J. H. Edmonson, and J. N. Ingle [3]

Maytansine (MAYT), an ansa macrolide from the shrub *Maytenus ovatus*, has mitosis-inhibiting properties similar to the vinca alkaloids and a spectrum of antitumor activity in animal models (1,2). An early clinical trial identified the dose-limiting toxic effects as severe nausea, vomiting, diarrhea, and liver function abnormalities (3). This study assesses the role of MAYT in patients with advanced head and neck cancer.

MATERIALS AND METHODS

Thirty-one patients with measurable or evaluable advanced head and neck cancer participated in the study. There were 19 males and 12 females with a median age of 62 years. Twenty-four patients had squamous cell cancers of which approximately one half were grade 1 or 2. Other histologic types included one patient with mixed cell cancer and two each with cylindroma, adenocarcinoma, and mucoepidermoid salivary gland cancers. Indicator lesions were predominantly soft tissue. Most patients had received extensive surgical treatment and radiation therapy. All patients were acceptable candidates for cytotoxic treatment based on nutritional and hematologic status and were ambulatory outpatients. Eastern Cooperative Oncology Group performance scores were primarily 0 or 1 (19 patients), with the remaining scores being 2 or 3 (12 patients). Eighteen patients had had no prior chemotherapy. The remaining 13 patients who had received prior chemotherapy with one or more cytotoxic agents had, except for slightly worse performance scores, covariate distributions similar to those of previously untreated patients.

Drug Administration

MAYT was administered by rapid iv injection at a dose of 0.6 mg/m^2/day for 3 consecutive days. The first retreatment was scheduled for 3 weeks with subsequent re-treatment at 4-week intervals. Patients were removed from study at the first indication of disease progression.

Evaluation

Prior to initial treatment and each subsequent course, patients had a complete general medical evaluation, routine hematologic and chemical parameter studies, chest roentgenogram, and photographs of appropriate lesions.

An objective response was defined as a decrease of $\geq 50\%$ in the product of the longest perpendicular diameter of the most clearly measurable indicator lesion. There must have been no new areas of malignant disease or deterioration in weight, symptoms, or performance score. Progressive disease was defined as a $> 25\%$ increase in any indicator lesion. Objective stability was defined as a $\leq 25\%$ increase in any indicator lesion. Patients with evaluable but not clearly measurable disease were evaluated using previously reported criteria (4). The time to progression was calculated from the first day of treatment to the date of progression or last followup.

RESULTS

Only one of 31 patients (3%) satisfied the criteria for an objective response. She was the oldest patient in the study (80 years of age) and had received no prior chemotherapy. Seventeen of 31 patients (55%) showed immediate disease progression following treatment, yielding a median time to progression for the entire study group (as well as for the subgroup of previously untreated patients) of 27 days. Six additional patients had disease progression after the second cycle leaving only 25% of the patients free of progression 2 months after the first administration of MAYT. Three patients, all having had no prior chemotherapy, are currently classified as stable at 65, 79, and 98 days. Median survival for the entire group was 134 days.

Toxic Effects

The toxic effects of MAYT were primarily gastrointestinal. Moderate to severe nausea and vomiting occurred in six of 31 patients. Diarrhea was moderate to

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severe in five of 31 patients. Three patients complained of a flu-type syndrome characterized by intense myalgias and arthralgias, and one of these patients has refused further treatment. Mild and transient leukopenia (2300, 2600, 3800, and 3900 cells/mm^3) occurred in four patients and thrombocytopenia (77,000 cells/mm^3) occurred in one. MAYT had no obvious hepatic or renal toxicity.

The one patient who responded to MAYT had a squamous cell carcinoma and gradually developed a state of confusion 2 weeks after the fifth cycle of MAYT at a total dose of 16 mg. An electroencephalogram, a computerized tomogram of the brain, a neurologic examination, and chemical determinations did not identify metastatic disease or metabolic imbalance, and medications could not be incriminated. Although the acute episode abated after several days, the patient has remained mentally dull during the 4 months since that time and is confined to a wheelchair primarily because of severe peripheral neuropathy which has persisted even though she has not received additional MAYT for the past 4 months. The patient now has evidence of hepatic metastasis 7 months from the start of treatment with MAYT.

DISCUSSION

We were unable to demonstrate substantial clinical benefit from MAYT for patients with advanced head and neck cancer. In fact, we can reject, at an $\alpha = 0.05$ level of significance, the hypothesis that the objective tumor response rate is at least 15% in similar patients with advanced head and neck cancer. Since most patients had disease progression within 1 or 2 months after starting MAYT, we cannot assess the chronic toxicity of the agent. However, the patient with the longest exposure to MAYT developed incapacitating neurologic symptoms which have been noted by others (5,6). MAYT, at the dose and schedule we utilized, cannot be recommended for further study in head and neck cancer. Our observations are consistent with other studies which show little therapeutic value from this agent for diverse solid tumors (5,6).

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Phase II Trial of Vindesine in Patients With Acute Leukemia [1,2,3]

Brief Reports and Preliminary Communications

Robert B. Sklaroff, Zalmen Arlin, and Charles W. Young [4,*]

SUMMARY

Vindesine was administered to 18 patients with acute leukemia who had failed conventional chemotherapy. Each course of therapy consisted of an iv bolus infusion at a dose of 1–2 mg/m^2 given daily × 5–10 days. Of 13 patients with acute lymphoblastic leukemia, two had partial remissions which lasted 2 and 3 months and five had minor responses. One of three patients with acute nonlymphoblastic leukemia and one of two patients with blastic crisis of chronic myelogenous leukemia each had a minor response. The data suggest that vindesine has activity in the treatment of acute leukemia.

[Cancer Treat Rep 63:2063–2065, 1979]

Vindesine is active against some transplanted murine leukemia strains (P388 and P1534) and ineffective against others (L1210, AKR, L5178, and C1498) (1,2). Because the antitumor spectrum of vindesine closely parallels that of vincristine, an agent of established clinical activity in leukemia and lymphoma, it is appropriate that vindesine be tested for remission induction in various hematologic malignancies.

In phase I evaluation, vindesine produced dose-limiting myelosuppression, mild to moderate neurotoxicity, alopecia, mucositis, acute flu-like symptoms, and local vascular injury (1,3,4). Mathé et al (5) reported that a continuous infusion of vindesine at a dose of 2 mg/m^2/day iv over 2 days induced six complete remissions (CRs) ("normal myelogram") and one partial remission (PR) ("less than 50% blast cells persisting in myelogram") in 15 children with acute lymphoblastic leukemia (ALL). Because responding patients were shifted to other regimens for maintenance of the remission, the French study (Mathé et al) did not provide data on remission duration. Krivit and Hammond (6), reporting for the Children's Cancer Study Group (CCSG), obtained three CRs in 35 children with ALL given weekly vindesine at a dose of 4 mg/m^2.

Dyke and Nelson (1) observed that vindesine had a considerably shorter plasma half-life than vincristine (β half-life, 0.822 versus 3.24 hours; γ half-life, 24.3 versus 144.0 hours) and a 3.5-fold more rapid clearance from the body. These considerations suggest the possible merit of a daily administration schedule for vindesine in disorders with rapid cell proliferation such as acute leukemia. Tan (7) has reported four remissions in six children with acute leukemia given daily vindesine at a dose schedule of 2 mg/m^2/day × 5 days at the Memorial Sloan-Kettering Cancer Center (MSKCC). The extended experience at MSKCC with vindesine in childhood acute leukemia will be communicated in a subsequent report; this communication reports the results of a phase II trial of vindesine in adult patients with acute leukemia in relapse after conventional chemotherapy.

MATERIALS AND METHODS

All patients (total, 18 patients) entered in this trial of vindesine had acute leukemia which had become refractory to conventional chemotherapy. Prior therapy had consisted of from two to five polydrug regimens; 15 of 18 patients had received vincristine. Vindesine was infused slowly over 5–10 minutes through the side arm of a free-flowing iv at a dose of 1–2 mg/m^2/day × 5 days (22 courses in 16 patients) or 1 mg/m^2/day × 10 days (seven courses in six patients). An adequate trial was defined as one producing therapeutic effect, or, in the absence thereof, evidence of dose-limiting toxicity.

CR was defined as the disappearance of all clinical evidence of leukemia on physical examination and peripheral blood, bone marrow, and biochemical evaluation for a minimum of 4 weeks; a patient with a CR must have felt well, except for minor treatment-related symptoms, dur-

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[3]Vindesine was supplied by Eli Lilly and Co, Indianapolis, IN.

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TABLE 1.—Patient characteristics and results of vindesine therapy for patients with leukemia

Characteristic	Disease type	
	ALL	ANLL/BCML
Total No. of patients	13	5
Median age in yrs (range)	21 (18–36)	38 (15–45)
Males/females	10/3	3/2
Performance status	40 (20–80)	60 (40–60)
Prior chemotherapy		
Median No. of drug regimens (range)	4 (2–5)	3 (2–5)
No. of patients with prior vincristine	13	2
Median dose in mg/m^2/day (range)	1.5 (1.0–2.0)	1.3 (1.0–2.0)
Median duration of courses in days (range)	5 (5–10)	5 (5–10)
Toxicity		
Median (range) wbc count nadir (cells/mm^3)	600 (400–1300)	2200 (600–4600)
Median (range) platelet count nadir (cells/mm^3)	11,000 (6000–84,000)	10,000 (5000–20,000)
No. of patients with—		
Mucositis	1	1
Neurotoxicity	1	2
Flu-like symptoms	2	1
Rash	0	1
Response		
No. of PRs	2	0
No. of MRs	5	2

ing this period. A PR indicated that the normal bone marrow status persisted for < 4 weeks or that the patient failed to achieve normal peripheral blood cell counts. A minor response (MR) was defined as an objective decrease either in circulating leukemic cells or marrow infiltration, as documented by at least two consecutive aspirates performed 1 week apart, or in leukemic skin nodules.

RESULTS

Patient characteristics and results of therapy are shown in table 1. Two (5-day) courses were given at 1.0 mg/m^2/day, 14 courses were given at 1.5 mg/m^2/day, and six courses were given at 2.0 mg/m^2/day. All patients entered in the study were adequate and evaluable for response. Vindesine produced evidence of therapeutic activity in seven of 13 patients with ALL; there were two PRs and five MRs. One PR was achieved after one course of vindesine at 1.5 mg/m^2/day × 5; it was maintained by a second, identical course and a course of 1.0 mg/m^2/day × 10. This PR persisted for 3 months overall. The other PR was achieved after one course of vindesine at 2 mg/m^2 × 5 consecutive days followed by three outpatient injections at the same dosage; it persisted for 2 months. The MRs lasted 8, 6, 6, 3, and 2 weeks, respectively.

A total of five patients in the series received multiple courses at 14–21-day intervals. This included three patients with antitumor effect and two patients without antitumor effect. Maximal benefit was achieved with the first vindesine course in the two patients with PR and in the one patient with MR; subsequent courses maintained the effect transiently. Two nonresponding patients received two and five courses in unsuccessful attempts to produce remission even though the initial course provided no therapeutic effect. Vindesine produced MR in one of three patients with acute nonlymphoblastic leukemia (ANLL) and in one of two patients with blastic crisis of chronic myelogenous leukemia (BCML); decreases in peripheral blast cells were 69,000 to 600/mm^3 and 85,000 to 800/mm^3, respectively. Neither type of responder showed significant marrow hypocellularity on the days of maximal peripheral blood response.

Vindesine caused significant hematologic toxicity; all ALL patients developed a granulocyte count of < 1300/

mm^3, but only those patients with ANLL and BCML who achieved MRs showed any decrease in peripheral granulocytes. In this series, daily vindesine produced less severe neurotoxicity than had been expected. Since 15 of 18 patients had received prior vincristine, it was impossible to detect subtle neurotoxicity; they had paresthesia and hyporeflexia prior to entry in the study. Paralytic ileus occurred in one patient who received 2 mg/m^2/day × 5 of vindesine.

DISCUSSION

Our results support the observations of Tan (7), Mathé et al (5), and the CCSG (6); vindesine is an active agent in patients with ALL. Our observed CR plus PR rate of 15% (two of 13 patients) is lower than that obtained by Tan (55%; six of 11 patients) and Mathé (47%; seven of 15 patients) but is similar to that obtained by Krivit and Hammond (9%; three of 35 patients). These differences could be due to a number of factors: small numbers in the present series, differing definitions for response, and variable intensity of prior treatment.

In the French series, vindesine was administered by infusion over 2 days during each cycle; the CCSG series received weekly vindesine at a dose of 4 mg/m^2; the pediatric MSKCC series remissions were obtained by bolus injections on both weekly and daily × 5 schedules. All lymphoblastic leukemia patients in this series had received heavy prior exposure to vincristine; they appeared to have been refractory to that drug's effects.

The relation of response to treatment dose is difficult to define in a small series. An examination of only the initial course of vindesine showed the following: a dose of 1.0 mg/m^2/day × 5 failed to produce a response in three patients; a dose of 1.5 mg/m^2/day × 5 produced one PR and four MRs in nine patients; and a dose of 2.0 mg/m^2/day × 5 produced one PR and two MRs in four patients. The dose of 2.0 mg/m^2/day × 5 also produced paralytic ileus in one patient and leukopenia of < 2000 cells/mm^3 for a median of 21 days.

The durations of remission in the CCSG report and in the present study were brief: 5, 6, and 8 weeks and 8 and 12 weeks, respectively. Mathé chose to attempt to maintain vindesine-induced remissions with conventional agents; we gave vindesine rather than trying to maintain response with drugs that had failed in the past. Since Mathé did not discuss duration of response and our responses were short-lived, no conclusion may be drawn as to how remissions might best be maintained.

We failed to obtain useful remissions in five patients with ANLL or BCML. In three patients the drug treatment even failed to lower circulating blast cell counts. Although these observations are consistent with the lower utility of vinca alkaloids in ANLL, more patients must be evaluated before a response rate for ANLL and BCML may be established.

This vinblastine analog does have activity in acute leukemia; therefore, the clinical observations support those in experimental tumor systems indicating similar tumor activity spectra for vindesine and vincristine. However, vinblastine itself as a single agent or in combination is reported to have produced four CRs and two PRs in 13 children with acute monocytic leukemia (9); based upon that report, the antileukemic activity of vinblastine itself may have been underestimated. Both of these agents deserve further trial in patients with acute leukemia.

Because of appropriate ethical constraints, it is not possible to examine a new vinca analog as initial therapy for acute leukemia where initial CRs in $> 90\%$ of patients with ALL and in $> 70\%$ of patients with ANLL are observed with current multiple-drug protocols. A relatively realistic assessment of the therapeutic potential of vindesine in these disorders might be obtainable by studying the drug's remission-induction capacity following patient relapse from polydrug therapy. At that time, the tumor cells may not yet have become fully vinca-resistant; furthermore, since the chance of a "cure" with conventional therapy has been reduced, the use of a single new drug, for induction purposes, would be more ethically acceptable.

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"Adriamycin Flare": A Skin Reaction Resembling Extravasation [1]

Brief Reports and Preliminary Communications

Nicholas J. Vogelzang [2,3,*]

SUMMARY

One hundred consecutive patients were prospectively observed for the development of a local skin reaction to Adriamycin. A frequency of 3% was noted. "Adriamycin flare" is possibly an allergic reaction and could be confused with Adriamycin extravasation.

[Cancer Treat Rep 63:2067–2069, 1979]

Adriamycin is an anthracycline antibiotic which has antineoplastic activity in a wide variety of malignancies (1). Cardiac and hematologic toxic effects are significant (2). Multiple skin reactions to Adriamycin have been described (3–5); the most serious of these reactions is extravasation in the subcutaneous tissue with subsequent necrosis. Contractures and chronic painful ulcers can result, and skin grafts (6) may be necessary. The identification of an Adriamycin infiltration demands the immediate discontinuation of the infusion. However, a local allergic reaction to Adriamycin can be confused with an infiltration and can cause intense anxiety in the chemotherapist.

CASE REPORT

A 56-year-old male with metastatic squamous cell carcinoma of the lung was receiving therapy with cyclophosphamide, Adriamycin, methotrexate, and procarbazine (7). Cyclophosphamide, Adriamycin, and methotrexate were given iv on Days 1 and 8 and procarbazine was given orally on Days 1–10; the cycle was repeated every 28 days.

During the third course of therapy, after methotrexate and cyclophosphamide had been infused, 30 mg of Adriamycin was given by slow iv push. A broad erythematous streak, which followed the course of the vein for 6–8 cm, developed as the infusion was completed. Urticaria was observed within 5 minutes after the infusion. The patient did not complain of pain, pruritus, or tenderness. The erythema and urticaria subsided within 30 minutes. The patient had three subsequent infusions of Adriamycin and no further reactions were noted.

METHODS

In order to define the frequency of the Adriamycin reaction, a daily record was kept of all outpatients who received Adriamycin. Adriamycin was infused by medical oncology nurses who recorded the presence or absence of "Adriamycin flare," which was defined as erythema, induration, and/or pruritus following the course of a vein and persisting after the cessation of the infusion. This definition was prominently posted and was discussed with each nurse. All Adriamycin was given by slow iv push, using a two-syringe technique, with care taken to obtain a blood reflux after each 5–10 mg. The nurses recorded the amount and lot number of the Adriamycin as well as other drugs given.

RESULTS

From September 21, 1977, to January 10, 1978, 183 doses of Adriamycin were given to 100 patients. The average dose was 48.5 mg (range, 20–100 mg).

Adriamycin flare occurred in six different patients (table 1). All patients had received Adriamycin previously and five patients continued to receive the drug without further incident. In all of the patients the flare subsided within 30 minutes, without sequelae. According to medical histories, none of the patients had experienced a prior flare.

To determine any relation of the flare to the manufacturing process, the lot numbers were reviewed. Lot 344 of Adriamycin was associated with the flare in two patients; however, no flares were observed in 72 other patients who also received lot 344.

One patient who experienced a flare had had a similar reaction to mechlorethamine. Another patient did not experience a flare but did complain of diffuse pruritus

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TABLE 1.—Patient characteristics

Patient No.	Age	Sex	Neoplasm type	Adriamycin dose (mg)	Concomitant agents
1	56	M	Lung cancer	20	Cyclophosphamide, methotrexate, and procarbazine
2	40	M	Hodgkin's disease	20	Vinblastine, DTIC, and bleomycin
3	72	M	Hepatoma	40	None
4	19	M	Hodgkin's disease	45	Vinblastine, DTIC, and bleomycin
5	58	F	Breast cancer	20	Cyclophosphamide, methotrexate, and procarbazine
6	69	M	Lung cancer	50	*cis*-Dichlorodiammine-platinum (II)

immediately after the injection. The pruritus was similar to that associated with his prior penicillin allergy.

There were no cases of anaphylactic shock or Adriamycin extravasation.

DISCUSSION

These results indicate that the Adriamycin flare is rare, occurring with only 3% of administered doses. Its benign nature is obvious.

There have been three prior reports describing Adriamycin flare. Etcubanas and Wilbur (8) first described the reaction as observed in 13 of 60 children receiving Adriamycin (60 mg/m^2) as an iv push. In their series, there was one case of nonfatal anaphylaxis. Antihistamines were not uniformly successful in preventing the reaction. Souhami and Feld (9) reported ten patients with local urticaria following a slow iv injection of Adriamycin. Ostrowski (10) documented one case of Adriamycin flare which occurred during an iv drip administration.

The first question raised by this study relates to the etiology of the flare. An immediate hypersensitivity reaction (type I) has been suggested and is supported by one case of anaphylaxis following the local reaction. The lack of response to histamine (H_1) antagonists and the failure of subsequent injections to uniformly produce the flare argue against an allergic cause. Other possibilities, such as drug interactions or contaminants, seem very remote. A more likely possibility is an actual molecular extravasation through the vascular endothelium. Reich and Bachur (3) described a laboratory worker who exhibited contact dermatitis secondary to Adriamycin treatment. The patient noted red-orange fluorescence in the area of dermatitis while under an ultraviolet light. This was attributed to the known fluorescence of DNA-Adriamycin complexes. Adriamycin flare may be a transient binding of the drug to local tissues, and a biopsy of the area with evaluation under ultraviolet light may document Adriamycin binding.

The second issue raised by this study is the method of Adriamycin administration. The package insert from Adria Laboratories, Inc (Wilmington, DE) suggests that "local erythematous streaking may be indicative of too rapid administration," and they recommend that the drug be administered over 3–5 minutes into the tubing of a rapidly running iv solution. It is possible that the rate of administration may lead to the development of "local erythematous streaking." However, the iv push method of administration produces a 3% incidence of local erythematous streaking, and the method may reduce the incidence of Adriamycin extravasation, especially in patients who are agitated or nauseated, or in those who have small veins (6).

In summary, the Adriamycin flare is a rare and benign event unrelated to Adriamycin extravasation. Further studies, including biopsy material, are needed to establish the etiology of the Adriamycin flare.

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cis-Dichlorodiammineplatinum(II) in Metastatic Soft Tissue Sarcomas [1]

Brief Reports and Preliminary Communications

C. P. Karakousis,* O. A. Holtermann, and E. D. Holyoke [2,3]

SUMMARY

cis-Dichlorodiammineplatinum(II) was given to 13 patients with metastatic sarcomas following failure in two previous chemotherapy protocols. Two of these patients had complete regression which lasted 6 months in one patient and is still present after 8 months in the other. One patient had a > 50% regression which lasted 5 months. Three patients had disease stabilization for 2 months each. _cis_-Dichlorodiammineplatinum(II), as a single agent, was well-tolerated with no significant renal or auditory toxicity.

[Cancer Treat Rep 63:2071–2075, 1979]

cis-Dichlorodiammineplatinum(II) (DDP) has been studied in patients with a wide variety of tumors, with best responses seen in testicular, ovarian, and bladder carcinomas and squamous cell carcinomas of the head and neck (1–3).

There is no information, with the exception of isolated case reports, on the effect of DDP in soft tissue sarcomas.

The following is a report of the activity of DDP, as a single agent, in metastatic sarcoma when given as tertiary chemotherapy following failure with a combination of cyclophosphamide, vincristine, Adriamycin, and DTIC (CYVADIC protocol [4]) and high-dose methotrexate.

MATERIALS AND METHODS

Thirteen patients with metastatic soft tissue sarcomas were given chemotherapy with DDP. There were four patients with leiomyosarcomas, two with fibrosarcomas, two with hemangiopericytomas, and one patient each with neurofibrosarcoma, rhabdomyosarcoma, malignant fibrohistiocytoma, liposarcoma, and clear cell sarcoma.

There were eight males and five females and their mean age was 43 years (range, 18–66). Six of these patients had lung metastases only. Five patients had pulmonary as well as other metastatic sites. One patient had brain, abdominal, and multiple soft tissue metastases (clear cell sarcoma). One patient had paraspinal soft tissue involvement, intra-abdominal metastases, and liver metastases (leiomyosarcoma that had originated in the uterus). Seven of these patients received DDP at a dose of 100 mg/m^2 diluted in 2 liters of 5% dextrose in 0.33 normal saline with 18.75 g of mannitol and 20 mg of Lasix in each liter. The 2 liters were infused in 6 hours and the treatment was repeated every 4 weeks. Six patients received DDP at a dose of 1 mg/kg diluted in 2 liters of 5% dextrose in 0.33 normal saline with 18.75 g of mannitol in each liter. The 2 liters were infused in 6 hours and the treatment was repeated weekly four times and then every 4 weeks.

Oral hydration was encouraged prior to each treatment. If there was clinical evidence of dehydration, iv fluids were also given. Besides cbc, BUN, and serum creatinine, creatinine clearance and an audiogram were obtained prior to each treatment.

RESULTS

Of the seven patients who received DDP at a dose of 100 mg/m^2 every 4 weeks (three leiomyosarcomas, two hemangiopericytomas, one malignant fibrohistiocytoma, and one neurofibrosarcoma), one patient with a metastatic paraspinal leiomyosarcoma mass (measuring 15 × 20 cm) and a lower abdominal mass had complete clinical regression of palpable tumors lasting 6 months (figs 1 and 2); this patient had previously failed with the CYVADIC protocol but had a temporary regression with high-dose methotrexate. Another patient with malignant fibrohistiocytoma metastatic to the lungs had 60% regression lasting 5 months; he had previously shown disease stabilization while receiving CYVADIC and high-dose methotrexate. Two patients had disease stabilization for 2 months each, while three patients had disease progression.

These patients received an average of 3.5 courses. The treatment was well-tolerated without renal or auditory toxicity (one patient had only slight high-frequency hear-

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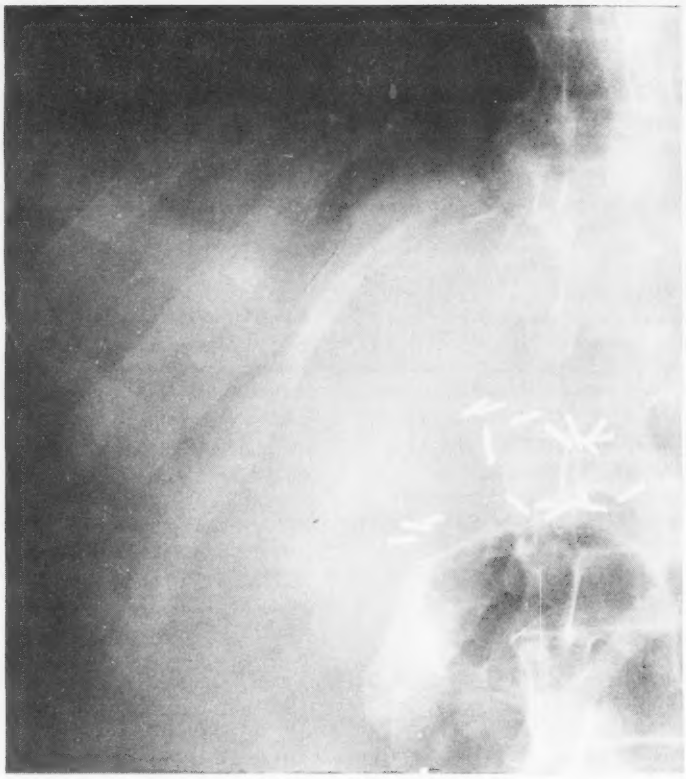

FIGURE 1.—This patient with metastatic leiomyosarcoma had a bulging mass in the right paraspinal dorsolumbar area measuring 15 × 20 cm. This tumor had destructed the lateral border of the 12th thoracic and 1st lumbar vertebrae.

ing loss), but all patients had moderately severe nausea and vomiting.

Six patients received DDP at a dose of 1 mg/kg/week × 4, then every 4 weeks. All of the tumors (two fibrosarcomas, one leiomyosarcoma, one liposarcoma, one clear cell sarcoma, and one rhabdomyosarcoma) in these patients had measurable parameters. The patient with rhabdomyosarcoma presented with a 0.8-cm nodule in the left lower lobe, as seen by chest roentgenogram (fig 3), after two median sternotomies for resection of pulmonary metastases; this nodule disappeared completely after initiation of DDP and the patient remains recurrence-free at 8 months (fig 4). Previously, this patient had failed with CYVADIC and later with high-dose methotrexate. Another patient with leiomyosarcoma had stabilization of her pulmonary metastases for 2 months, but treatment with DDP was discontinued because of decreasing creatinine clearance (in two instances < 60 ml/minute) although her BUN and serum creatinine remained normal; four patients had disease progression.

Of the six patients given DDP at a dose of 1 mg/kg as outlined above (average, four doses), one patient had a decrease in creatinine clearance to < 60 ml/minute although her BUN and serum creatinine remained normal; after discontinuation of DDP treatment her creatinine clearance returned to normal levels. Two of these patients had mild high-frequency hearing loss. All patients had moderately severe nausea and vomiting.

None of the 13 patients had evidence of myelosuppression as a result of the treatment with DDP.

DISCUSSION

DDP, either as a single agent (5,6) or in combination (7), has been found to be effective therapy for advanced osteogenic sarcoma.

There is limited information on the effect of DDP in soft tissue sarcomas. No responses were noted in one study among eight patients with soft tissue sarcomas

FIGURE 2.—Restoration of the lateral border of the same vertebrae, still evident 5 mos after starting DDP therapy. This was accompanied by the complete disappearance of the soft tissue component.

given DDP at a dose of 20 mg/m² iv daily × 5, repeated every 4 weeks (8). Using a weekly outpatient schedule of DDP (40 mg/m²), no responses were noted in three patients with sarcomas (9). There was no response in a patient with synovial sarcoma who was given DDP at a dose of 100 mg/m² iv, repeated every 3 weeks (10). In addition, no responses were observed in a phase I study of five patients with soft tissue sarcomas (11) or in two children with rhabdomyosarcomas (12) who were given DDP. One response was noted among three patients with sarcomas treated with combination chemotherapy of Adriamycin and DDP (13).

Because of the lack of a systematic study of the effect of DDP in soft tissue sarcomas, a phase II study has been recently proposed to include a total of 29 patients (14).

A variety of dose schedules have been used with DDP as a single agent (15,16).

The most important toxic effect of DDP is renal failure which is largely prevented with the use of adequate iv hydration, mannitol, and furosemide (11,17). Furosemide has usually been added, if necessary, in order to maintain a urine output of 150 ml/m²/hour during DDP infusion, and is usually given in the course of 6–8 hours (6). Although irreversible kidney damage can occur (5), impairment of renal function is usually reversible after discontinuation of treatment (18). Myelosuppression may also be observed, with the nadir occurring at Days 10–14, but is usually not severe (19). Hearing loss has been reported in 3% of patients (20). Peripheral neuropathy has been described rarely (21).

Of the 13 patients described in this report, only one had evidence of impaired renal function manifested with a low creatinine clearance (< 60 ml/minute) despite normal BUN and serum creatinine. After discontinuation of treatment, creatinine clearance returned to normal in 4 weeks. Three patients had mild high-frequency hearing loss. All patients had moderately severe nausea and vomiting. Three of the patients had objective regression (two

FIGURE 3.—Nodule in the left lower lobe best seen at the 16-cm tomographic cut; it was also evident on the lateral view of the plain radiograph.

complete regressions) of 5, 6, and 8 months' duration (the last regression still with no evidence of recurrence). In this small series, the objective response rate of 23% observed with DDP in metastatic soft tissue sarcomas, after failure with two previous chemotherapy protocols, is encouraging and suggests further evaluation of DDP in these tumors.

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FIGURE 4.—Disappearance of the nodule 1 mo after starting DDP therapy (16-cm tomographic cut).

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Phase II Study of Alternating Cytoreductive and Cycle-Active Combination Chemotherapy for Metastatic Breast Cancer [1,2]

Brief Reports and Preliminary Communications

Charles L. Vogel,* Neil H. Love, Dawn R. East, Melvin R. Moore, and Richard V. Smalley [3]

SUMMARY

Nineteen females with metastatic breast cancer (73% with visceral disease) treated with cyclophosphamide, Adriamycin, and 5-fluorouracil (CAF) cytoreduction followed by, and then alternated with, an intensive "cycle-active" regimen were evaluable. Sixteen of the 19 (84%) patients responded to CAF prior to initiation of the cycle-active regimen, and median remission duration for CAF responders was 50 weeks. The regimen was tolerable and the remission rates and durations were at least as good as those seen with CAF alone. This regimen is now being compared with CAF in a randomized trial of the Southeastern Cancer Study Group.

[Cancer Treat Rep 63:2077–2079, 1979]

The combination of cyclophosphamide, Adriamycin, and 5-fluorouracil (CAF) is one of the most effective cytoreductive regimens in the treatment of metastatic breast cancer, yielding response rates of 43%–82% in reported series (1–4); however, the complete remission rates remain low (14%–20%) and the remission durations are relatively short (32–40 weeks). The present study was designed to see whether the use of an intensive cycle-active regimen after CAF cytoreduction, and alternating with CAF during remission maintenance, would increase the complete remission rates and prolong the remission durations over those reported for CAF alone.

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PATIENTS AND METHODS

Twenty-four females with metastatic breast cancer who had received no prior cytotoxic chemotherapy had a pretreatment evaluation including history and physical examination, cbc, platelet count, liver and renal function tests, carcinoembryonic antigen, chest roentgenogram, ECG, liver and bone scans, and roentgenograms of bone scan-positive areas. Response to therapy was assessed according to published guidelines (5) and remission duration and survival were calculated from the date of entry in the study.

The chemotherapeutic regimen is shown in table 1. Chemotherapy was continued for a maximum of ≤ 1 year if a total dose of 450 mg/m^2 of Adriamycin was reached. Antitumor response was evaluated at Week 6, again at Week 9, and then after each 9-week course of therapy by physical examination, blood cell tests, and roentgenograms. Cbcs were done weekly until a tolerable dose of CAF was established, and then before each dose of CAF. Cbcs were also done before each dose of the cycle-active regimen, CAMELEON (cytosine arabinoside [ara-C], methotrexate, leucovorin, and vincristine). Doses of CAF were modified downward for marked myelosuppression, while doses of methotrexate and ara-C remained constant in the face of myelosuppression but with doubling of the number of doses of leucovorin being administered to a maximum of eight doses.

RESULTS

Nineteen of the 24 patients completed the initial 9 weeks of therapy and were considered evaluable for remission induction. The remaining five patients were considered unevaluable because of patient refusal due to gastrointestinal toxicity from CAF (three patients) or CAMELEON (one patient) and noncompliance (one patient). The characteristics of the evaluable patients are shown in table 2. Fourteen of 19 (73%) of the patients had visceral metastatic disease and ten of 19 (52%) had liver and/or lymphangitic lung disease.

TABLE 1.—CAF-CAMELEON regimen

Drug regimen	Drug and schedule	Route	Drug given on week—												
			First course								Second course				
			0	1	2	3	4	5	6	7	8	9	12	15	16	17
CAF																
Cyclophosphamide	500 mg/m² q21days	Iv push	×	–	–	×	–	–	–	–	–	×	×	–	–	–
Adriamycin	50 mg/m² q21days	Iv push	×	–	–	×	–	–	–	–	–	×	×	–	–	–
5-Fluorouracil	500 mg/m² q21days	Iv push	×	–	–	×	–	–	–	–	–	×	×	–	–	–
CAMELEON																
Vincristine	1.4 mg/m² on Day 1	Iv push	–	–	–	–	–	–	×	×	×	–	–	×	×	×
Methotrexate	30 mg/m² q6hrs × 4 on Day 1	Oral	–	–	–	–	–	–	×	×	×	–	–	×	×	×
Leucovorin	25 mg (total) q6hrs × 4 on Days 2 and 3	Oral	–	–	–	–	–	–	×	×	×	–	–	×	×	×
Ara-C	300 mg/m² on Day 2	Iv push	–	–	–	–	–	–	×	×	×	–	–	×	×	×

TABLE 2.—Patient characteristics (19 patients)

Characteristic	No. of patients
Mean age in yrs (range)	53 (30–73)
Menopausal status	
Premenopausal	5
Postmenopausal	14
Performance status (Karnofsky)	
> 70%	10
40%–70%	8
< 40%	1
Disease-free interval	
< 1 yr	14
1–5 yrs	3
> 5 yrs	2
Estrogen receptors	
(+)	2
(–)	8
(?)	9
No. of involved metastatic sites	
1–2	11
3+	8
Dominant metastatic site	
Soft tissue	3
Bone	2
Visceral	14*
Prior hormonal therapy	
No	13
Yes	6

*Includes liver (7 patients), nodular lung (4 patients), and lymphangitic (3 patients).

Sixteen of 19 (84%) evaluable patients responded to CAF, with a median time to response of 6 weeks (range, 3–12). There were five complete responses (26%) and 11 partial responses among 19 patients. Two patients failing CAF induction had partial responses (each lasting 17 weeks) to CAMELEON alone. Attempts to return to CAF after CAMELEON response resulted in renewed disease progression in these two patients. The patient failing CAF or CAMELEON had chest-wall disease as the only metastatic site. Since one of our major objectives was to see if the cycle-active regimen prolonged CAF remissions, remission duration was evaluated for 14 of 16 CAF responders. The two patients considered unevaluable were removed from the study early for geographic and financial reasons. The median remission duration was 49.9 weeks (range, 19–70) for the 14 evaluable patients. The median survival duration was 82.5 weeks (range, 26–141+), with six patients still alive.

CAF toxicity was comparable to that of previous reports (1–4) and CAMELEON was well-tolerated. There were no drug-related deaths and only one of 24 patients was removed from the study because of CAMELEON toxicity. Eighty-five courses of CAF and 173 courses of CAMELEON were evaluable for hematologic toxicity. Thrombocytopenia and anemia were insignificant problems. Granulocytopenia was generally encountered with full-dose CAF, and most patients required dose de-escalation. The mean total dose of CAF actually given to evaluable patients was 77% of the calculated dose. Only 11% of CAMELEON courses were associated with granulocytopenia. Nineteen percent of CAF doses after CAMELEON courses had to be delayed because of CAMELEON-induced toxicity. Only 9% of the projected total weekly number of CAMELEON courses could not be given because of toxicity. Nausea and vomiting was common with CAF and also after ara-C on Day 2 of a CAMELEON cycle. Stomatitis (secondary to CAF) was a severe problem in only one patient. Vincristine was the most troublesome drug in the regimen, causing severe jaw or abdominal pain in three patients and mild to moderate paresthesias in eight. Early in the study, five patients received vincristine weekly during the 2 weeks between courses of CAF and CAMELEON, and three of these patients developed foot drop causing these in-

terim vincristine doses to be dropped in all other patients. Seven of 19 evaluable patients received < 50% of the projected vincristine dose. There were no drug-related deaths.

DISCUSSION

Currently, efforts at increasing chemotherapy-induced complete remission rates and prolonging remission durations in breast cancer have focused on the addition of immunotherapy (4) or hormonal therapy (6,7) or alternating mutually non-cross-resistant combinations (8,9). Advances in the treatment of other human cancers have been claimed for regimens formulated to take advantage of changes in cell-cycle kinetics of tumor cells (10,11). One of these, the COMA regimen (cyclophosphamide, vincristine, methotrexate, and ara-C) for diffuse histiocytic lymphoma (10), was based on unpublished observations of Bertino (personal communication) in L1210 leukemia suggesting synergism between methotrexate and ara-C and curative scheduling, with cycle-active therapy given after cytoreduction. In the CAF-CAMELEON regimen, the most effective cytoreductive regimen for breast cancer (CAF) was used instead of cyclophosphamide, and Berd's cycle-active scheduling was adopted as our CAMELEON regimen because it was found to be both effective and tolerable in his study (10). Histiocytic lymphoma and metastatic breast cancer share common sensitivities to cyclophosphamide, methotrexate, and vincristine and a resistance to single-agent ara-C. Few kinetic studies in metastatic breast cancer and histiocytic lymphoma have been published. In those that have, wide variations from patient to patient have been noted (15 references are available from the authors upon request). It is interesting, however, that 40%–50% of patients with metastatic breast cancer have tumor volume doubling times of < 25 days (12), a finding not too dissimilar from that published for lymphomas in general (13).

The CAF-CAMELEON program produced tolerable toxicity, but lower doses of vincristine will be used in future studies. The scheduling of CAMELEON is rigorous and requires considerable patient motivation for optimal compliance. Although only a pilot study, the response rate of 84% (25% complete responses) is at least as good as that achieved with CAF alone. The median remission duration of 50 weeks for CAF responders is encouraging since 73% of these patients had visceral metastatic disease.

The CAF-CAMELEON regimen is currently being tested against CAF alone in a controlled, randomized trial of the Southeastern Cancer Study Group in females with visceral metastases. Even if the CAF-CAMELEON regimen proves superior in this trial, improvement could be due to the use of two mutually non-cross-resistant combinations, or to prolongation of the duration of Adriamycin therapy, or to the hypothesized cell-kinetic rationale. Kinetic studies using flow microfluorometry in patients receiving this regimen are now in progress in an effort to address the last possibility.

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Phase II Evaluation of Dianhydrogalactitol in Advanced Head and Neck Carcinomas [1,2,3]

Brief Reports and Preliminary Communications

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SUMMARY

Twenty-eight patients with advanced squamous cell carcinomas and adenocarcinomas of the head and neck were treated with dianhydrogalactitol at a dose of 25 mg/m² iv on each of 5 consecutive days monthly. All patients except one had received previous surgical and/or radiotherapeutic treatments and 12 had received previous chemotherapy. Disease progression (or symptomatic deterioration) occurred in nine patients after the initial course and in eight others after two courses. In 11 patients, the disease remained stable during at least three courses; however, no instances of objective regression of disease were observed during treatment with dianhydrogalactitol despite significant toxic effects in most patients.

[Cancer Treat Rep 63:2081–2083, 1979]

After the definition of a tolerable 5-day intermittent schedule for dianhydrogalactitol in man (1), we decided to study this agent in the treatment of advanced head and neck carcinomas. Dianhydrogalactitol, like dihalohexitols such as dibromodulcitol (2) and dibromomannitol (3), has been regarded as an alkylating agent; however, it is chemically unlike most of the standard alkylating agents, which are not very effective against head and neck carcinomas. Thus, in dianhydrogalactitol we hoped to identify a more effective alkylating agent for tumors of this type. In addition, early indications of activity against squamous cell carcinomas of lung origin (4) were somewhat encouraging because of the histologic similarity between these tumors and many carcinomas of head and neck origin.

MATERIALS AND METHODS

Twenty-eight patients with histologically proven inoperable advanced carcinomas of the head and neck were treated with dianhydrogalactitol. Before patients were entered in the study, adequate renal, hepatic, and bone marrow functions were assured (creatinine < 1.5 mg/dl; no increase in direct-reacting bilirubin; wbc count of $> 4100/\mu l$; and platelet count of $> 130,000/\mu l$). Twenty-seven of the patients had received extensive surgical and/or radiotherapeutic treatments and 12 had also received previous chemotherapy (table 1). Eighteen of the patients had squamous cell carcinomas, one had a lymphoepithelioma, six had adenocarcinomas, one had a mucoepidermoid tumor, and two had mixed salivary gland tumors. All patients had estimated survival expectations of at least 2 months, and no radiotherapy or cytotoxic drug treatment had been given to any patient within 4 weeks prior to the beginning of dianhydrogalactitol treatment.

Patients were given dianhydrogalactitol at a dose of 25 mg/m² by rapid iv injection on Days 1–5 of each monthly cycle. Treatment was continued in each case until disease progression was observed, and doses were modified where necessary according to wbc and platelet count nadirs. Objective disease regression was defined as a $\geq 50\%$ decrease in the product of the longest perpendicular diameters of measurable lesions without progression in the size of any lesion or the appearance of any new lesion. When disease was evaluable but not measurable, agreement between two observers was required to define the definite decrease in tumor size. Disease stability was defined as a $< 25\%$ increase or a $< 50\%$ decrease in the size of the measured lesions without the appearance of any new lesions. Disease progression was defined as the appearance of any new lesion or an increase of $\geq 25\%$ in the product of perpendicular diameters of any measured lesion.

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TABLE 1.—Phase II evaluation of dianhydrogalactitol in advanced head and neck carcinomas: patient characteristics

Sex	Age	Site of tumor origin and histology	Performance status*	Previous therapy	No. of courses of dianhydrogalactitol	Best objective response	Toxic effects
M	56	Ear canal, grade 3 squamous cell carcinoma	2	Surgery, radiotherapy, methotrexate, cyclophosphamide	1	Progression	Nausea and vomiting
F	54	Tonsil, grade 3 squamous cell carcinoma	3	Surgery, radiotherapy	3	Stability	Alopecia, leukopenia, thrombocytopenia
M	61	Base of tongue, grade 2 squamous cell carcinoma	1	Surgery, radiotherapy	1	Progression	Leukopenia, thrombocytopenia
F	64	External ear, grade 3 squamous cell carcinoma	1	Surgery, radiotherapy, methotrexate	1	Progression	Stomatitis, alopecia
M	56	Larynx and pyriform sinus, grade 3 squamous cell carcinoma	1	Surgery, radiotherapy	2	Stability	Stomatitis, leukopenia, alopecia
F	73	Tongue, grade 1 adenocarcinoma (cylindroma)	1	Radiotherapy	6	Stability	Leukopenia, thrombocytopenia
M	28	Nose and maxillary sinus, grade 3 adenocarcinoma	1	Radiotherapy	2	Stability	Leukopenia, thrombocytopenia
M	41	Nasopharynx, grade 3 squamous cell carcinoma	3	Radiotherapy	1	Symptomatic deterioration	None immediately
F	69	Larynx, grade 3 squamous cell carcinoma	0	Surgery, radiotherapy	3	Stability	Leukopenia, thrombocytopenia
M	62	Base of tongue, grade 4 squamous cell carcinoma	2	Surgery, radiotherapy	1	Symptomatic deterioration and death	Leukopenia, septic shock (lethal)
M	51	Larynx (extrinsic), grade 2 squamous cell carcinoma	1	Surgery, radiotherapy	1	Progression	Stomatitis
M	59	Gingiva, grade 2 squamous cell carcinoma	1	Surgery, radiotherapy, methotrexate + bacillus Calmette-Guérin	1	Progression	None
M	56	Buccal mucosa, grade 2 squamous cell carcinoma	0	Surgery, radiotherapy, methotrexate	2	Stability	None
M	59	Floor of mouth, grade 3 squamous cell carcinoma	1	Surgery, radiotherapy, methotrexate + bacillus Calmette-Guérin	4	Stability	Leukopenia, thrombocytopenia
M	57	Salivary gland, grade 1–2 malignant mixed tumor	1	Surgery	1	Progression	None
M	54	Tongue, grade 2 squamous cell carcinoma	1	Surgery, radiotherapy, bacillus Calmette-Guérin	2	Stability	Leukopenia
M	64	Unknown site, grade 4 squamous cell carcinoma	0	None	5	Stability	Leukopenia, thrombocytopenia
M	79	Larynx (intrinsic), grade 3 squamous cell carcinoma	1	Surgery, radiotherapy	2	Stability	None
M	44	Nasopharynx, grade 4 lymphoepithelioma	1	Radiotherapy	1	Symptomatic deterioration	Nausea and vomiting
M	51	Parotid, grade 3 adenocarcinoma	2	Surgery, radiotherapy	5	Stability	Leukopenia, thrombocytopenia
M	47	Nasopharynx, grade 4 squamous cell carcinoma	1	Radiotherapy	5	Stability	Leukopenia, thrombocytopenia
F	49	Ethmoid sinus, grade 3 squamous cell carcinoma	2	Surgery, radiotherapy, bleomycin-Adriamycin-methotrexate	2	Stability	Leukopenia, nausea and vomiting
F	65	Sublingual gland, grade 2 adenocarcinoma (cylindroma)	1	Surgery, 5-fluorouracil, bleomycin-Adriamycin-methotrexate	10	Stability	Leukopenia
M	60	Parotid, grade 1 adenocarcinoma	1	Surgery, radiotherapy, bleomycin-Adriamycin-methotrexate	13	Stability	Leukopenia

TABLE 1.—Phase II evaluation of dianhydrogalactitol in advanced head and neck carcinomas: patient characteristics—Continued

Sex	Age	Site of tumor origin and histology	Performance status*	Previous therapy	No. of courses of dianhydrogalactitol	Best objective response	Toxic effects
M	71	Soft palate, grade 3 squamous cell carcinoma	3	Surgery, radiotherapy, bleomycin-Adriamycin-methotrexate	2	Stability	None
F	36	Parotid, grade 1 adenocarcinoma	2	Surgery, bleomycin-Adriamycin-methotrexate	7	Stability	Leukopenia, nausea and vomiting
F	49	Parotid, grade 1 mucoepidermoid carcinoma	1	Surgery, radiotherapy, bleomycin-Adriamycin-methotrexate	3	Stability	Leukopenia, thrombocytopenia
F	58	Parotid, mixed tumor	1	Surgery, bleomycin-Adriamycin-methotrexate	2	Stability	None

*Eastern Cooperative Oncology Group.

RESULTS

All of the 28 treated patients are described in table 1. Nine patients experienced disease progression (or symptomatic deterioration) following the initial course of dianhydrogalactitol. The other 19 patients experienced disease progression after receiving from two to 13 courses of treatment. In 11 of these patients, the disease remained stable for at least three courses of treatment; however, six of these 11 patients had relatively slow-growing tumors of salivary gland type. No objective regression of disease occurred during treatment with dianhydrogalactitol. Median survival after the start of treatment was approximately 26 weeks (range, 2–76+). Nausea and vomiting were commonly observed following dianhydrogalactitol treatment, and significant stomatitis was observed in three patients. Median wbc and platelet count nadirs were approximately 3500/μl (range, 500–9400/μl)and 158,000/μl (range, 41,000–569,000/μl) respectively following the first course of dianhydrogalactitol. Seventeen patients developed leukopenia (wbcs < 4100/μl) and ten patients also developed thrombocytopenia (platelets < 130,000/μl) at some time during treatment. Two patients died prior to the expected time of initial posttreatment re-evaluation. One of these deaths was due to sepsis which was apparently secondary to drug-induced leukopenia.

DISCUSSION

Dianhydrogalactitol produced no tumor regression in these 28 patients with advanced head and neck carcinomas. Although disease stability was observed in 11 of the patients for at least 3 months, six of these 11 had relatively slow-growing tumors of salivary gland type; thus, our observation of disease "stability" is of doubtful therapeutic importance. As indicated by the drug toxicity observed, the doses were generally adequate to produce significant biologic effects. Although 12 of these patients had received previous antitumor chemotherapy, we believe that this study completes the therapeutic evaluation of dianhydrogalactitol as a single agent in advanced head and neck carcinomas.

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Treatment of Blast Cell Crisis of Chronic Myeloid Leukemia With ICRF-159 (Razoxane)[1]

Brief Reports and Preliminary Communications

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SUMMARY

Eleven patients with myeloid blast cell crisis and two patients with lymphoid blast cell crisis of chronic myeloid leukemia were treated with ICRF-159. Two of the patients with myeloid blast cell crisis achieved partial bone marrow remission and survived for 8+ and 18 months. One of the patients with lymphoid blast cell crisis reverted to the chronic phase of chronic myeloid leukemia after therapy with ICRF-159 in combination with prednisolone. The only significant toxic effect was myelosuppression.

[Cancer Treat Rep 63:2085–2087, 1979]

The majority of patients with chronic myeloid leukemia (CML) undergo terminal blastic transformation. Except in patients with lymphoblastic characteristics who may respond to vincristine and prednisone (1,2), the blastic phase is extremely refractory to treatment, the quality of life poor, and the median survival time about 3 months (3). Only two of 25 patients with myeloid blast cell crisis reported by Janossy et al (4) achieved remission (2 and 5 months) following combination chemotherapy, and the overall median survival time was 2 months. One patient, however, survived beyond 1 year. He failed to respond to intensive chemotherapy but responded to treatment with the cytostatic agent ICRF-159 (5) and survived for 18 months from the diagnosis of blast cell crisis. Encouraged by this response, a study was initiated to evaluate the activity of ICRF-159 in CML blast cell crisis.

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MATERIALS AND METHODS

Thirteen consecutive patients with CML blast cell crisis (table 1) were treated at St. Bartholomew's Hospital. Four had failed to respond to treatment with regimens incorporating vincristine and prednisolone. All patients fulfilled the criteria for blast cell crisis proposed by Beard et al (3), and the type of blast cell crisis was defined on the basis of cell morphology, reactivity of blast cells with anti-acute lymphocytic leukemia (anti-ALL) serum, and terminal transferase enzyme (Tdt) levels (4).

Drug administration.—ICRF-159 was administered orally in doses ranging from 150 to 750 mg/m^2/day in divided doses every 8 hours for 5 days. Courses were repeated at 2–4-week intervals. All patients received allopurinol.

Assessment of response.—Complete remission was defined as either a return to the bone marrow appearance of chronic phase CML or to a normal appearance with $< 5\%$ blast cells. Partial remission was defined as a reduction of $> 50\%$ in marrow blast cells plus a decrease of $> 50\%$ in organomegaly.

RESULTS

Eleven patients with myeloblastic cell morphology, failure to react with anti-ALL serum, and undetectable levels of Tdt were classed as myeloid blast cell crisis. The blast cells of patient 12 were lymphoblastic in type and 68% reacted with anti-ALL serum. Insufficient cells were available for Tdt estimation. Patient 13 had both lymphoblastic and myeloblastic cells, 80% reacted with anti-ALL serum, and the Tdt level was 9.6 units/10^8 cells (normal = < 2 units/10^8 cells [4]). These two patients were classed as lymphoid blast cell crisis.

Response

Two of the 11 patients with myeloid blast cell crisis achieved partial remission. Patient 1 presented without a chronic phase and failed to respond to a combination of vincristine, prednisolone, cytosine arabinoside, 6-thioguanine, and Adriamycin. Five months after diagnosis he started treatment with ICRF-159 and received

TABLE 1.—Patient characteristics

Patient No.	Age (yrs)	Duration of chronic phase (mos)	Primary therapy* (blast cell crisis)	Response	Bone marrow blast cells†	ICRF-159 dose	No. of courses	Response‡	Survival (mos)
1	35	0	V+P+TG+CA +ADR	None	35%	150–750 mg/m²/day × 5	26	PR	18
2	59	33	V+P	None	80%	150 mg/m²/day × 3	2	NR	3
3	20	30	V+P+TG+CA	None	44%	150–200 mg/m²/day × 5	2	NR	4
4	34	29	V+P	None	47%	300–600 mg/m²/day × 5	5	NR	3
5	42	51	—	—	26% (+ 25% promyelocytes)	300–600 mg/m²/day × 5	9	NR	5
6	17	8	—	—	54%	300 mg/m²/day × 5	1	NR	<1
7	60	9	—	—	69%	300–500 mg/m²/day × 5	3	NR	3
8	60	20	—	—	90%	300–400 mg/m²/day × 5	3	NR	1
9	30	96	—	—	30%	300 mg/m²/day × 5	2	NR	2
10	37	19	—	—	56%	300–500 mg/m²/day × 5	8	PR	8+
11	64	52	—	—	80%	300 mg/m²/day × 5	2	NR	<1
12§	17	19	—	—	82%	300 mg/m²/day × 5¶	1	CR	5
13	15	0	—	—	90%	300 mg/m²/day × 5	2	NR	2+

*V = vincristine, P = prednisolone, TG = 6-thioguanine, CA = cytosine arabinoside, ADR = Adriamycin.
†Immediately prior to ICRF-159 therapy.
‡PR = partial remission, NR = no response, CR = complete remission.
§Lymphoid blast cell crisis.
¶Plus prednisolone (40 mg/day) for 11 days starting on Day 17 of the ICRF-159 course.

a total of 26 courses at 2-week intervals. His symptoms resolved following the first course, and a 13-cm liver became impalpable and his spleen decreased in size from 20 to 8 cm over a period of 6 months. The wbc count, initially 488,000/mm³, stabilized at 5000–10,000/mm³ after a dose of 625 mg/m²/day × 5 was reached, and marrow blast cells fell from 35% to 12%. Remission was maintained for 12 months and the patient survived for 18 months.

Patient 10 presented in blast cell crisis after a 19-month chronic phase. Following two courses of ICRF-159, his marrow blast cells fell from 56% to 11% and his peripheral blood cell counts improved, with a rise in hemoglobin from 9.7 to 14 g/100 ml and a rise in platelet count from 14,000 to 118,000/mm³. At present, treatment with 500 mg/m²/day × 5 at monthly intervals maintains the wbc count at 10,000–20,000/mm³. The marrow blast cells continue at < 25%. Patients 1 and 10 were managed on an outpatient basis, returned to full-time employment, and remained symptom-free for 12 and 7+ months. A third patient with myeloid blast cell crisis (patient 5) showed a clinical resolution of symptoms including arthralgia, bone pain and sweats, and a decrease in spleen size. There was, however, no change in marrow appearance. He remained well and was able to work for 5 months until death from a cerebral hemorrhage due to drug-induced thrombocytopenia. One patient with lymphoid blast cell crisis (patient 12) achieved complete remission (reversion to chronic phase CML) following one course of ICRF-159 in combination with prednisolone. The peripheral blood cell counts began to deteriorate after 3 months but the patient refused further treatment and died at 5 months. There were no responses after failure to respond to ICRF-159 in four patients treated subsequently with regimens including vincristine, prednisolone, and Adriamycin.

Toxicity

The only significant toxic effect was myelosuppression. Drug-induced neutropenia (fall to < 1000 cells/mm³ or > 50% reduction) occurred in six patients (46%) and was associated with a non-fatal septicemia in one. Thrombocytopenia (fall to < 100,000 cells/mm³ or > 50% reduction) occurred in eight patients (61%) and was associated with hemorrhage in five. This was fatal in two cases (patients 5 and 11), neither of whom were supported with platelet infusions. Patients 1 and 10 developed tolerance to this

side effect in spite of further increases in doses. Other side effects included alopecia in four patients receiving $\geq$ 500 mg/m^2/day of ICRF-159 and mild nausea in two patients.

DISCUSSION

ICRF-159 has been reported to have only minimal activity in acute myelogenous and acute lymphoblastic leukemias (6). The evidence from this study, however, suggests that the drug may sometimes provide effective palliation in CML blast cell crisis in a manner similar to that of busulfan in chronic phase CML. The drug was well-tolerated, disease control was satisfactory, and the quality of life was excellent in two patients with myeloid blast cell crisis, although only partial hematologic remission was achieved. It is unclear whether the remission in the patient with lymphoid blast cell crisis was induced by ICRF-159, prednisolone, or the combination.

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Phase II Trial of VP-16-213 in the Treatment of Advanced Squamous Cell Carcinoma of the Cervix and Adenocarcinoma of the Ovary: A Gynecologic Oncology Group Study[1]

Brief Reports and Preliminary Communications

Robert E. Slayton, William T. Creasman, William Petty, Brian Bundy, and John A. Blessing[2,3,*]

VP-16-213 is a semisynthetic podophyllotoxin derivative. Like its congener, VM-26, VP-16-213 inhibits cells entering mitosis, possibly by an effect on DNA synthesis (1). In contrast to other podophyllotoxins which are spindle poisons, VM-26 and VP-16-213 appear to block entry of cells into mitosis, acting during the late S and G_2 phases of the cell cycle (2,3).

Both agents have antitumor activity, with VP-16-213 being the most effective plant product against L1210 leukemia. Responses have been observed in patients with lymphoma (4,5), leukemia (5), brain tumors (6), lung cancer (7), etc. Toxic effects include leukopenia, thrombocytopenia, alopecia, nausea, vomiting, and diarrhea, all of which are generally reversible when therapy is discontinued. Headache, fever, and hypotension have been recorded infrequently. Phase II studies were initiated by the Gynecologic Oncology Group (GOG) in March 1977 to evaluate the effectiveness of VP-16-213 in gynecologic cancer.

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[2]This study was performed by the Gynecologic Oncology Group and was supported in part by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare. The following members participated in the study: *R. E. Slayton* (Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL); *W. T. Creasman* (Duke University Medical Center, Durham, NC); *W. Petty* (University of Oregon Health Sciences Center, Portland); and *B. Bundy* and *J. A. Blessing* (Gynecologic Oncology Group, Roswell Park Memorial Institute, Buffalo, NY). Participating institutions and their grants are: New York Medical College (CA-12483); Bowman Gray School of Medicine (CA-21946); Georgetown University (CA-16938); Rush-Presbyterian-St. Luke's Medical Center (CA-12485); University of Mississippi Medical Center (CA-13633); University of California at Los Angeles (CA-13641); University of North Carolina (CA-19189); University of Nebraska (CA-23084); University of California Medical Center at Irvine; Duke University Medical Center (CA-12534); University of Oregon Health Sciences Center; and Gynecologic Oncology Group (CA-19189).

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MATERIALS AND METHODS

Females with advanced or recurrent tumors of the genital tract, which were histologically confirmed, progressing, and not amenable to further surgery, radiotherapy, or chemotherapy, were eligible for this trial. All patients had measurable disease. Neither ascites nor pleural effusion alone was considered measurable for the purposes of this study. Measurements obtained by ultrasonography were accepted if sharply defined. A performance status in the range of 30–100 (Karnofsky scale) was required.

A granulocyte count of $> 1500/\mu l$ and a platelet count of $> 100,000/\mu l$ were required (later amended to require a granulocyte count of $> 2000/\mu l$ and a platelet count of $> 150,000/\mu l$ for patients previously treated with Adriamycin and/or an alkylating agent). In addition, a BUN of < 25 mg/100 ml, a serum creatinine of < 1.5 mg/100 ml, a bilirubin of < 1.0 mg/100 ml, and an SGOT of < 50 IU/liter were required.

Standard response criteria were used. A complete response was defined as the disappearance of all gross evidence of disease for at least 1 month. A partial response (PR) was defined as a $\geq 50\%$ reduction in the product of the largest diameter and the perpendicular of all measurable lesions for a period of at least 1 month. Progressive disease was defined as a $\geq 25\%$ increase in the product of the largest diameter and the perpendicular of all measurable lesions or the appearance of any new lesion within 3 months of study. Stable disease was defined as a response not meeting any of the above criteria.

Hematologic toxicity is defined in table 1. Patients who received at least one course of treatment and lived for another 3 weeks were considered evaluable for response. All patients who received at least one course were considered evaluable for toxicity regardless of subsequent survival.

VP-16-213 (100 mg/m^2) was administered iv over a 45-minute period, diluted in 150 ml of normal saline on Days 1, 3, and 5. The cycle was repeated every 28 days. A cbc was done weekly. BUN, creatinine, SGOT, and bilirubin levels and tumor measurements were repeated every 4

TABLE 1.—Drug dose modification according to hematologic toxicity

Lowest wbc count grade*	Lowest platelet count grade†			
	0	1	2	3–4
0	Increase 1 level	No change	Decrease 1 level	Decrease 2 levels
1	No change	No change	Decrease 1 level	Decrease 2 levels
2	Decrease 1 level	Decrease 1 level	Decrease 1 level	Decrease 2 levels
3–4	Decrease 2 levels	Decrease 2 levels	Decrease 2 levels	Decrease 2 levels

*Wbc count: grade 1 = < 4000/mm^3, grade 2 = < 3000/mm^3, grade 3 = < 2000/mm^3, and grade 4 = < 1000/mm^3.

†Platelet count: grade 1 = < 150,000/mm^3, grade 2 = < 100,000/mm^3, grade 3 = < 50,000/mm^3, and grade 4 = < 25,000/mm^3.

weeks. Treatment courses were repeated every 4 weeks if permitted by bone marrow recovery. Doses were adjusted according to the schedule shown in table 1 using dose levels of 50, 75, 100, 125, and 150 mg/m^2.

Any delay of > 4 weeks in resuming treatment required a one-level dose reduction. A one-level dose escalation was permitted after dose reduction if no hematologic toxicity was observed at the lower level. Doses were reduced by two levels for nausea, vomiting, or diarrhea requiring iv fluids or hospitalization.

RESULTS

Squamous Cell Carcinoma of the Cervix

Forty-one patients with squamous cell carcinoma of the cervix were entered in the study. Two of these patients were found to be ineligible and eight were found to be unevaluable (two were never treated, three had major chemotherapy violations, and three had inadequate data). Thus, 31 patients were considered evaluable, one of whom had insufficient nadir counts for evaluation of toxicity. No responses were noted. Nineteen patients had stable disease, while 12 had increasing disease (table 2). Median survival was significantly longer ($P = 0.001$) for patients with stable disease (table 3). Age, time to recurrence, prior surgery, prior radiotherapy, grade, and performance status were not related to type of response. A history of prior chemotherapy was more common in patients with increasing disease (seven of 12) than in patients with stable disease (seven of 19).

TABLE 2.—Response rates for squamous cell carcinoma of the cervix

Response category	No. of patients	% of patients
Stable disease	19	61.3
Increasing disease	12	38.7
Total	31	100.0

TABLE 3.—Survival by response category for squamous cell carcinoma of the cervix

Response category	Survival (mos)	
	Median	Range
Stable disease	6.19	(1.5–13+)
Increasing disease	1.75	(0.5–9.5)
Totals	3.55+	(0.5–13+)

Of 19 patients with stable disease, three had doses increased by two levels and two had doses decreased by one level. The median number of courses for these patients was three. None of 12 patients with increasing disease had a dose modification; the median number of courses for this group of patients was one. The overall median number of courses was two (range, one to nine).

Of 30 patients evaluable for toxicity, there were two with grade 1, four with grade 2, and one with grade 3 wbc count toxicity. There were five patients with grade 1 platelet count toxicity. Gastrointestinal toxicity was seen in nine patients; it was mild or moderate in eight. One patient had severe toxic effects requiring hospitalization and iv fluids, and elevated BUN levels were observed in two patients.

Adenocarcinoma of the Ovary

Thirty-six patients with adenocarcinoma of the ovary who had failed previous chemotherapy were entered in the study. One of these patients was found to be ineligible and eight were found to be unevaluable (five had major protocol violations, two had inadequate data, and two

TABLE 4.—Response rates for epithelial tumors of the ovary

Response category	No. of patients	% of patients
Partial response	2	8.3
Stable disease	9	37.5
Increasing disease	13	54.2
Total	24	100.0

TABLE 5.—Survival by response category for epithelial tumors of the ovary

Response category	Survival (mos)	
	Median	Range
Partial response	8+	(5–11+)
Stable disease	6+	(3–11+)
Increasing disease	3.2	(1–4.5)
Total	4.3	(1–11+)

were never treated). Of the remaining 27 patients, three had inadequate documentation of tumor size for a determination of response, while two others had insufficient nadir counts for evaluation of toxicity. Thus, 24 patients are evaluable for response while 25 are evaluable for toxicity. There were two PRs in this group. Nine patients had stable disease and 13 had increasing disease (table 4). Of the two PRs, one was achieved with three courses and lasted for < 2 months and the other was achieved with five courses and lasted for 3 months. Median survival was not different for responding patients and for patients with stable disease, but was significantly shorter ($P = 0.01$) for patients with increasing disease (table 5).

Age, time to recurrence, histologic grade, prior surgery, prior radiotherapy, and performance status were not related to type of response.

Dose was reduced by one level in four patients and by two levels in one patient; none of these patients responded. The median number of courses was four for patients with stable disease and one for patients with increasing disease. Overall, the median number of courses was two (range, one to eight).

Of 25 patients evaluable for toxicity, there were eight with grade 0, three with grade 1, eight with grade 2, three with grade 3, and three with grade 4 wbc toxicity. Eleven patients had no gastrointestinal toxicity, six had mild toxicity, seven had moderate toxicity, and one had severe toxicity. There were 14 patients with grade 0 platelet toxicity, four with grade 1, three with grade 2, three with grade 3, and one with grade 4. Elevations of BUN and creatinine were observed in two patients. Other toxic effects included mild itching and alopecia. Hypercalcemia, hyperuricemia, neurotoxicity, and cardiovascular side effects were not observed, and there were no drug-related deaths.

DISCUSSION

Only a few patients with gynecologic tumors, all with ovarian cancer, were included in earlier phase II trials of VP-16-213. Dombernowsky et al (4) observed no response in a single patient with ovarian cancer using a 5-day course every 3 weeks. Using a similar schedule, Jungi and Senn (8) noted four responses among six patients with advanced ovarian cancer, with only one, however, achieving a > 50% reduction in tumor size. Falkson et al (9) reported a PR in three of 12 patients receiving the drug orally. Edmonson et al (10) observed no responses among 14 patients receiving a dose of 120 mg/m^2 iv on Days 1, 3, and 5 every 5 weeks.

Using a dose of 140 mg/m^2 of VP-16-213 iv on Days 1, 3, and 5 every 4 weeks, Eagan et al (7) observed tumor responses with mild to moderate hematologic and gastrointestinal toxic effects in patients with advanced adenocarcinoma and squamous cell carcinoma of the lung, none of whom had received prior chemotherapy. Since most patients entering the GOG trial had received extensive prior radiotherapy, chemotherapy, or both, the dose used for initial treatment was 100 mg/m^2 iv on Days 1, 3, and 5. Even with this dose reduction, severe hematologic toxicity was seen in five patients, necessitating a protocol modification which required an initial granulocyte count of 2000/μl and a platelet count of 150,000/μl for patients previously treated with an alkylating agent alone or combined with Adriamycin. Giving the same total dose over 5 days, Jungi and Senn (8) could find no correlation between hematologic toxicity and antitumor response.

Cumulative myelosuppression was not observed in the few patients who received more than four cycles.

VP-16-213 has minimal activity against ovarian cancer at the current dose and schedule and insignificant activity against squamous cell carcinoma of the cervix in phase II trials. It is not recommended for phase III trials in ovarian cancer or squamous cell carcinoma of the cervix.

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Pulmonary Hyaline Membrane Disease Occurring in the Course of VM-26 Therapy[1]

Brief Reports and Preliminary Communications

James R. Commers* and John F. Foley[2,3]

SUMMARY

During treatment with VM-26 a 20-year-old patient with metastatic medulloblastoma developed an acute, fulminating pulmonary illness. Open lung biopsy revealed advanced alveolar hyaline membrane formation with interstitial round cell infiltrate. This is the first reported case of podophyllotoxin-related pulmonary disease.

[Cancer Treat Rep 63:2093–2095, 1979]

Busulfan was initially reported to cause interstitial pulmonary fibrosis in 1961 (1). Since that time a number of other cytotoxic agents, including methotrexate, cyclophosphamide, bleomycin, and the nitrosoureas, have been associated with the development of pulmonary disease. To our knowledge this is the first reported case of acute, hyaline membrane disease occurring during therapy with VM-26.

CASE REPORT

A 16-year-old Caucasian male was found to have a posterior fossa mass lesion in March 1974. Cerebellar craniectomy with Torkildsen ventricular cisternostomy revealed an unresectable medulloblastoma in the brainstem at the floor of the fourth ventricle. Postoperatively, the patient received 5040 rads over a 5½-week period to the tumor and local structures.

The patient did well until October 1975 when he was re-admitted with numbness and weakness in the lower extremities. Myelography demonstrated a complete spinal subarachnoid block in the lumbar region. At emergency decompressive laminectomy, diffuse tumor studding of the entire subarachnoid space was found. In the postoperative period the patient was treated with irradiation, receiving 4600 rads to the entire spinal axis and 4320 rads to previously untreated areas of the cranium over a 5-week period. BCNU therapy was initiated.

In the ensuing 10 months, neurologic improvement continued to the point that the patient could resume farming full-time. BCNU was administered every 6–8 weeks from October 1975 to July 1978 to a total cumulative dose of 2120 mg (1115 mg/m^2). During this time the patient suffered no pulmonary symptoms and his chest roentgenogram remained normal. In September 1978 new neurologic deficits led to a computerized axial tomographic scan of the brain which demonstrated recurrent tumor in the upper cerebellar occipital area. Myelography revealed almost complete obstruction of flow at the foramen magnum.

Symptomatic increased intracranial pressure developed and therapy with dexamethasone was begun. Therapy was changed to weekly VM-26, with the patient receiving an 1800-mg (947-mg/m^2) cumulative dose from September 10, 1978, to November 28, 1978. During that period his neurologic status improved and the dexamethasone dose was tapered to 1.5 mg three times daily.

In early December 1978, 3 days after his last dose of VM-26, the patient was admitted with a 72-hour history of progressive dyspnea and 2 hours of worsening obtundation. Examination revealed an unresponsive, deeply cyanotic, tachypneic individual. Examination of the chest revealed no evidence of consolidation or effusion. Arterial blood gases, with the patient breathing oxygen at 4 liters/minute by nasal cannula, were pH 7.52, PCO_2 25 mm Hg, and PO_2 25 mm Hg. On 100% F_IO_2 the PO_2 improved only to 54 mm Hg. Chest roentgenogram showed advanced diffuse interstitial and alveolar infiltrates in all lobes. An open lung biopsy revealed numerous alveoli lined with thick hyaline-like fibrinous material. Additionally, marked interstitial infiltrate with lymphocytes, plasma cells, histiocytes, and occasional giant cells was present (fig 1). Interstitial fibrosis was absent. Gomori methenamine silver stains were negative for *Pneumocystis carinii* and fungi. Bacterial, mycobacterial, and fungal cultures were negative.

Despite vigorous postoperative supportive measures, improvement in pulmonary function did not occur and

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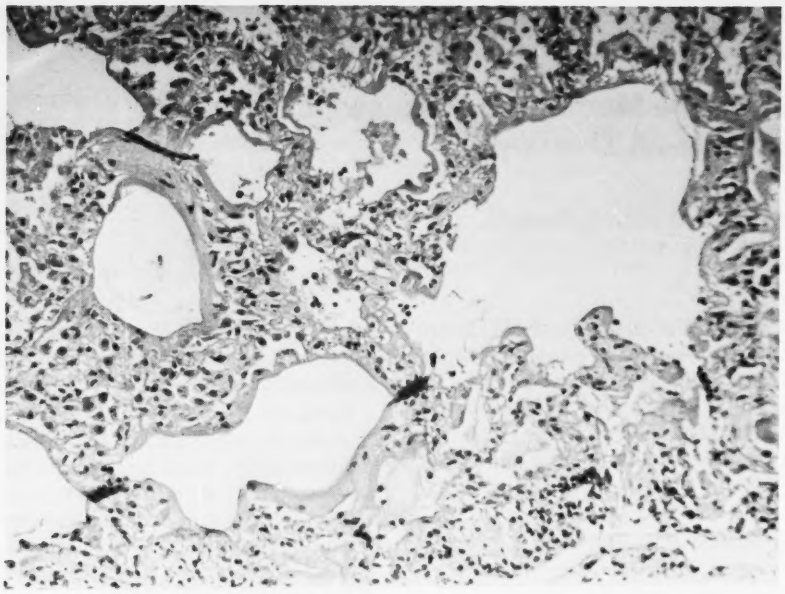

FIGURE 1.—Photomicrograph of lung biopsy demonstrating alveolar hyaline membranes and interstitial round cell infiltrate (hematoxylin-eosin stain, × 160).

just before death the patient developed a fulminating staphylococcal pneumonia. Postmortem examination revealed diffuse metastatic medulloblastoma covering the cerebellum, brainstem, cerebrum, and spinal cord in the subarachnoid space. The right and left lungs were diffusely consolidated, weighing 1000 and 850 g respectively. Microscopic examination revealed diffuse bronchopneumonia and *Staphylococcus aureus* was grown from a lung culture. Additionally, in some areas the alveolar spaces were filled with hyaline membranes. There was no evidence of interstitial fibrosis. Gomori methenamine silver stains again revealed no fungal or parasitic organisms. Viral cultures obtained from lung tissue obtained during the autopsy were negative.

DISCUSSION

Recent reviews (2–5) have defined the scope of cytotoxic drug-induced pulmonary disease. Alkylating agents, particularly busulfan and cyclophosphamide, along with methotrexate and bleomycin most commonly have been associated with pulmonary side effects. More recently, the nitrosoureas, particularly BCNU, have been implicated in the pathogenesis of interstitial pulmonary fibrosis (6–11). A single report has correlated methyl-CCNU with the appearance of interstitial pulmonary fibrosis (12).

In all of the 17 adult patients with BCNU-induced pulmonary toxicity reported so far in the literature, the striking pathologic diagnosis was interstitial pulmonary fibrosis, either patchy or diffuse. In only a single patient, reported by Bellot and Valdiserri, were pulmonary hyaline membranes noted (11). These were present at autopsy only after the patient had received high concentrations of inspired oxygen for a period of days and had not been noted in an open lung biopsy prior to such therapy.

In the patient reported here the temporal relationship between therapy with VM-26 and the development of an acute pulmonary syndrome suggests a causative role for the drug. Of note, the patient had previously been treated with the nitrosourea BCNU. It seems doubtful that the BCNU was responsible for the pulmonary disorder for three reasons. First, at no time during the course of BCNU therapy did the patient suffer respiratory symptoms. His chest roentgenogram remained normal during that period. Second, the acute pulmonary event occurred some 5 months after discontinuance of the BCNU. The literature is not entirely clear as to when patients develop pulmonary disease in the course of BCNU therapy, but from the available data, it seems likely that the chance of developing BCNU-induced pulmonary toxicity lessens with the passage of time after cessation of drug treatment. Finally, the histopathologic picture of diffuse and focal interstitial pulmonary fibrosis reported in association with nitrosoureas is much different from that of hyaline membrane formation with interstitial round cell infiltrate seen in our patient. Hyaline membrane formation has also been reported in association with pulmonary disease caused by methotrexate, bleomycin,

cyclophosphamide, and busulfan, none of which were received by this patient. Other known causes of adult hyaline membrane disease, including prolonged inhalation of high concentrations of oxygen, viral, bacterial, and chemical pneumonias, and "uremic" lung, could not be implicated in the present case at the time of the initial lung biopsy.

Etiologic mechanisms involved in the production of the pulmonary disorder presented here are unclear. The absence of peripheral eosinophilia and alveolar eosinophilic infiltrates suggests that hypersensitivity was not involved. It seems most likely that a direct toxic effect of VM-26 was responsible for the dramatic pulmonary hyaline membrane disease observed. With the expanding use of the podophyllotoxin derivatives VM-26 and VP-16-213 in clinical cancer care, cognizance of potential pulmonary toxicity is of distinct importance.

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Phase II Evaluation of Vindesine in Patients With Advanced Colorectal Carcinoma [1,2,3]

Brief Reports and Preliminary Communications

David S. Carroll, Richard J. Gralla,* and Nancy E. Kemeny [4,5]

Vindesine is a semi-synthetic vinca alkaloid prepared from vinblastine. In experimental tumor models, it has shown a broad spectrum of antineoplastic activity which differs somewhat from that exhibited by other vinca alkaloids (1). In phase II trials, vindesine has shown activity against lung cancer (2,3), esophageal carcinoma (4), melanoma (5), breast cancer (5), and testicular carcinoma (5,6). Activity has also been noted in Hodgkin's disease (7,8), acute lymphocytic leukemia, and chronic myelocytic leukemia (7). Between July 1978 and February 1979 we conducted a phase II trial with vindesine at the Memorial Sloan-Kettering Cancer Center in patients with measurable metastatic colorectal carcinoma.

MATERIALS AND METHODS

Vindesine was given by weekly iv injection at an initial dose of 3 mg/m^2. Subsequent doses were increased by 0.5 mg/m^2 in the absence of toxicity and were attenuated in the presence of significant myelosuppression or neurotoxicity. The drug was not given when the wbc count was $< 2500/mm^3$. An adequate trial was defined as a total dose of ≥ 10 mg/m^2 of vindesine and at least four weekly injections. A partial remission was defined as a $\geq 50\%$ decrease in the sum of the products of the largest perpendicular diameters of all measurable disease for at least 1 month. A minor response was defined as a $> 25\%$ decrease in measurable tumor, and patients who demonstrated a $\leq 25\%$ change in tumor size were considered to have stable disease.

The diagnosis of colorectal adenocarcinoma for the 25 patients who were entered in the study was confirmed by our pathology department. The median age was 58 years (range, 42–74 years) and the median Karnofsky performance status was 80 (range, 60–100). There were 13 males and 12 females. The median time from diagnosis of metastatic disease until entry in this study was 12 months. Seven patients (28%) had received prior radiotherapy and all patients had received prior chemotherapy. Nineteen patients (76%) had received prior therapy with vincristine as part of a polydrug combination regimen which also contains methyl-CCNU and 5-fluorouracil. Sites of metastatic disease were: lung (12 patients); liver (18); pelvic mass (three); vulva (one); bone (one); and subcutaneous (one). Twelve patients (48%) had more than one site of metastatic disease.

RESULTS

Twenty patients received an adequate trial. Two of the five unevaluable patients died and three chose not to receive further chemotherapy before adequate total doses were administered. For the 20 evaluable patients the median total dose of vindesine was 15 mg/m^2 (range, 10–41.5 mg/m^2) and the median number of doses was five (range, four to 17). No partial remissions were seen. One patient, with hepatomegaly and a colloid shift observed on liver scan, had a minor response with a decrease in liver size and normalization of scan during vindesine therapy. Two patients had stable metastatic pulmonary disease while receiving eight and nine weekly doses of vindesine; they were treated every other week to reduce neurotoxicity, and received 5 and 7 months of vindesine therapy before developing enlargement of pulmonary nodes. Two additional patients had stable hepatic metastases while receiving vindesine, and all other patients developed measurably progressive disease during the course of therapy.

Toxicity

All patients experienced some form of neurotoxicity; as table 1 indicates, paresthesias and constipation were the most common. Severe neurotoxicity resulting in prolonged paralytic ileus or marked weakness occurred in three patients (14%). Fever unassociated with infection was common (45%) during the 24–48 hours after vindesine administration. Hoarseness was observed in six patients;

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[3]Vindesine was supplied by Eli Lilly and Co, Indianapolis, IN.

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[5]We wish to thank Kathleen Trainor for preparation of the manuscript.

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TABLE 1.—Vindesine toxicity in 20 patients with colorectal carcinoma

Toxic effect	No. of patients (%)
Paresthesias	18 (90)
Alopecia	17 (85)
Weakness	13 (65)
Constipation	11 (55)
Fever	9 (45)
Myalgia	9 (45)
Numbness	8 (40)
Hoarseness	6 (30)
Jaw pain	3 (15)
Paralytic ileus	2 (10)
Wbc count nadir (cells/mm^3)	
≥4000	1
3000–3999	8
2000–2999	10
<2,000	1
Hgb decrease (g/dl)	
0–1.9	10
2.0–2.9	4
3.0–3.9	6
Platelet count nadir (cells/mm^3)	
≥200,000	11
150,000–199,999	7
100,000–149,999	2
<100,000	0

two of these patients had pharyngitis but the hoarseness in the other four was not associated with any evidence of inflammation. In the two patients who underwent laryngeal examination, no abnormalities were identified. Moderate leukopenia was produced in most patients and this was often dose-limiting. The median wbc count nadir was 2400/mm^3 (range, 1600–4100/mm^3), and the median platelet count nadir was 240,000/mm^3 (range, 110,000–317,000/mm^3).

DISCUSSION

Twenty-five patients with metastatic colorectal carcinoma were treated with weekly injections of vindesine. Weakness, leukopenia, paresthesias, and constipation seen in other phase II studies using a weekly treatment schedule (3–6) were also the major side effects of therapy in this trial. One minor response constituted the only demonstrable therapeutic activity. It can be predicted from the present trial that vindesine, administered weekly at this dose in previously treated patients with advanced colorectal carcinoma, has a true major response rate of $< 15\%$ ($P = 0.05$) (9).

The results of the present study may have been influenced by the prior exposure of 76% of the patients to vincristine. Although Mathé et al (7) noted no cross-resistance with vincristine in patients with hematologic malignancies, Reynolds et al (6), in a phase II trial of vindesine in patients with germ cell tumors, observed responses only in those patients who were the least heavily pretreated with vinblastine. In view of these considerations, further studies of vindesine in colorectal carcinoma may be indicated in patients who have not received prior chemotherapy.

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Continuous Central Venous Access in Patients With Acute Leukemia [1,2,3]

Brief Reports and Preliminary Communications

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The treatment of adult acute leukemia requires aggressive supportive care of the patient during induction chemotherapy used to achieve complete remission. Maintaining iv lines to provide this support is a constant problem. Indwelling central venous catheters have been used safely for years for home parenteral hyperalimentation (1). This study was designed to investigate the safety of the same type of catheter used in patients with adult acute leukemia during induction chemotherapy; all patients had granulocytopenia, most had thrombocytopenia, and more than half had systemic infections.

METHODS

Patient population.—Thirty-two continuous central venous access lines (eight Broviac, 18-bore, and 24 Hickman, 16-bore) were placed in 25 adult patients with acute nonlymphocytic leukemia. Initially, catheters were placed only in patients lacking peripheral access. Beginning in February 1978, all patients with acute nonlymphocytic leukemia undergoing chemotherapy for induction of remission had lines inserted. Patients received standard intensive induction therapy with daunorubicin and cytosine arabinoside (2) or one of a variety of regimens used in patients who have relapsed (5-azacytidine [3], COAP [4],[6] or daunorubicin and cytosine arabinoside in induction doses).

Catheter insertion and maintenance.—The catheters were placed with the same techniques used for insertion of continuous home parenteral hyperalimentation catheters (1,5). Preoperative platelet transfusions were given if the platelet count was $< 30{,}000/mm^3$. The catheter exit site on the chest wall was redressed every 48 hours using a strict aseptic technique similar to that used for parenteral hyperalimentation line dressings. If the patient was not receiving continuous medication or fluids, the catheter was capped with a male Luer lock cap. A heparin-containing flush (1000 units of heparin in 10 ml of normal saline) was given whenever blood passed in or out of the line (eg, with blood product transfusion, blood drawing, or blood backflow in the line) and whenever continuous iv fluid control was discontinued. Capped catheters were flushed daily (1). In order to prevent a catheter in use from clogging, it was necessary to ensure that fluid was continuously passing through the catheter. Therefore, the catheter was connected to a continuous infusion pump. Prior to administration of blood products, normal saline was run directly into the pump through the tubing and was continuously infused during the blood product infusion.

Uses of the catheter.—The catheter was used for blood drawing and for administration of multiple antibiotics, antifungal agents, iv push medications, electrolyte solutions, and all blood products (platelets, rbcs, and wbcs). Chemotherapy with agents that are not locally irritating was also given through the catheter. Vesicant chemotherapy was not given through the catheter in order to avoid the risk of instilling such chemotherapy into a catheter where subcutaneous leaks could have been present.

Home care.—The patient and his or her family were taught catheter dressing techniques and flush procedures while he or she was in the hospital. They were also given complete written instructions prepared by the nursing staff.

Catheter removal.—Catheter removal was an office procedure, done under local anesthesia.

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[5]We gratefully acknowledge the dedicated assistance of Drs. John H. Glick and Peter A. Cassileth in reviewing the manuscript, the Nursing Staff of the Oncology Study Unit, and Patricia A. Lafferty in preparing the manuscript.

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[6]COAP = cyclophosphamide, vincristine, cytosine arabinoside, and prednisone.

RESULTS

All patients received chemotherapeutic agents, antibiotics, fluids and electrolytes, rbcs, and platelets through the catheter. Some received antifungal agents and wbc transfusions. Peripheral iv lines were inserted for vesicant chemotherapy administration. Catheters were in place for a median of 4 weeks (range, 0.5–33).

Complications

Three of the first seven Broviac (18-bore) catheters developed leaks in their subcutaneous portions, which were felt to be induced by vigorous flushing during episodes of catheter clogging. With the subsequent use of the controlled-volume infusion, even during periods of blood product transfusion, the clogging disappeared and no further leaks occurred.

Thrombocytopenia, present in the majority of patients, never prevented catheter placement or removal. Preoperatively, after platelet transfusion if indicated, four patients had platelet counts $< 20{,}000/mm^3$, 13 had counts between 20,000 and $60{,}000/mm^3$, and 14 had counts $> 60{,}000/mm^3$. Local bleeding or hematoma formation at the insertion site was minimal in all but two patients whose counts were 9000 and $14{,}000/mm^3$ respectively. The latter patient had acute promyelocytic leukemia with disseminated intravascular coagulation at the time of catheter placement and a massive hematoma resulted.

Infection

All patients were granulocytopenic, with neutrophil counts of $< 500/mm^3$, for a median of 4 weeks (range, 1–13) while the catheters were in place. Fifteen of the 25 patients had systemic infections, but in only one case was the catheter tip itself infected. This infection cleared when the catheter was removed and appropriate antibiotics were given. The systemic infections included three cases of *Staphylococcus aureus*, two cases of *Staphylococcus epidermidis*, five cases of *Pseudomonas aeruginosa*, one case each of *Serratia*, *Enterobacteriaceae*, *Hemophilus parainfluenzae*, and *Escherichia coli*, one case of multiple anaerobes, one abdominal wound with bowel flora appearing systemically, and one perirectal abscess. There was one case of central nervous system *Mycobacterium flavescens*. Six patients had fungal infections. There were two cases of aspergillosis (one confined to the lung), one case of *Candida tropicalis*, and three cases of candidiasis (one systemic). Four patients had both bacterial and fungal infections. There were two cases of skin infections in the catheter exit site on the chest wall, both of which responded easily to appropriate systemic antibiotic therapy and did not require catheter removal. Fifteen patients died with a catheter in place; nine of these had an autopsy. In the nine autopsied cases, all of the lines were free of infection and thrombus, and the heart and blood vessels as well as the pulmonary vessels showed no abnormality related to the presence of the catheters. The lines of the patients not autopsied were not thought to be infected because premortem blood cultures obtained through them were negative. Ten catheters were removed while the patients were alive. One was the infected line and three were the leaking Broviac catheters mentioned above. The leaking lines and the other six lines were not infected or clotted at the time of removal. Eleven patients were discharged with lines in place; they maintained the catheters after discharge for a median of 8 weeks (range, 1–26). One patient left the hospital without permission with the catheter in place and therefore was not considered evaluable for this study. In the evaluable patients no catheters have clotted or have become infected in the home care period.

DISCUSSION

The continuous right atrial Silastic catheter appears to be safe in adult patients with acute leukemia undergoing intensive chemotherapy, in spite of the fact that they have had prolonged thrombocytopenia, prolonged granulocytopenia, frequent bacteremia, and occasional fungemia. Use of the catheters, in addition to presenting no greater risk to the patients, has the benefit of avoiding the unique problems associated with the usual peripheral iv lines. These include phlebitis from the needles or the solutions infused, multiple venipunctures, pain from products being infused, and limb immobilization. We have not identified the ideal material or insertion method for a continuous catheter in adult acute leukemia patients since our study was not designed to investigate these questions. We do feel that centers which use Silastic catheters for continuous home parenteral hyperalimentation can easily and safely adapt these catheters for use in adult acute leukemia patients to maintain continuous venous access.

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Rational Effective Medical Treatment of Skin Ulcers Due to Adriamycin[1]

Brief Reports and Preliminary Communications

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Adriamycin extravasation frequently results in ulceration at the site of infiltration. Characteristically, these ulcers have virtually no propensity to heal, even though the immediate milieu in which they exist is normal. They appear as punched-out lesions with slightly raised edges. The base is free of granulation tissue, and there is no evidence of extension of proliferating epithelium from the ulcer edge into the base (1). The blood supply surrounding the ulcer has been found to be histologically intact (1). Spontaneous healing is rarely seen; it may occur in lesions < 2 cm in diameter (2).

At the present time, conventional treatment consists of excision of the entire ulcer, including the base, followed by grafting. Direct grafting to the ulcer has not been successful (2).

The unique properties of the "Adriamycin ulcer" would lead one to believe that some substance exists within the ulcer which prevents healing. We postulate that this substance is an Adriamycin-DNA complex which has been described (3) and was shown to exist in animal models (4). Based upon this concept, a method of management was undertaken which would theoretically reduce the concentration of this complex.

PATIENTS, MATERIALS, AND METHOD

Five patients with a total of seven ulcers were treated. The ulcers varied in size from 75 to 5 cm^2 and ranged in age from 18 months to 3 weeks. The oldest ulcer was free of any overlying devitalized skin. Prior to initiating therapy, necrotic tissue was removed by covering the lesions with streptokinase and streptodornase gel. The lesions were kept moist with a saline dressing. Fresh enzyme was added two to three times daily, 24 hours a day, for about 1 week. The necrotic tissue could then be easily removed. A 0.5% solution of sodium hypochlorite was freshly prepared in the pharmacy. A template of the exact size of the base of the ulcer was cut from a dressing. This was soaked in the sodium hypochlorite solution and placed in the ulcer. The edge of the ulcer was lightly covered with petroleum jelly to prevent maceration of viable tissue by the solution. If pain occurred, a small amount of 2% lidocaine sterile solution was placed in the ulcer prior to applying the sodium hypochlorite dressing. The dressing was covered with a plastic wrapping and allowed to remain in place for about 8 hours. This was repeated for 5–7 consecutive days, whereupon it was discontinued for 5–7 days and then reinstituted. During the period of treatment between soaks, the patient washed the ulcerated area with clean water and applied a dry nonsticking absorbent dressing. Subsequently, after stopping the sodium hypochlorite soaks, a cross-linked dextran polymer (dextranomer) was applied liberally to the wound and covered by a dry dressing. Once a day the dextranomer was washed from the wound with water. This treatment was continued until granulation tissue appeared in the wound.

RESULTS

One patient died from her disease 3 weeks after the start of treatment. In the remaining four patients the six ulcers all underwent healing (fig 1). Frequently, there was a latency period of about 6 weeks before healing started. One patient who was receiving corticosteroids had a longer latency period. The initial phase of healing consisted of growth of epithelium from the edge of the ulcer into the base. Clinically, this was manifest as a change in the physical characteristics of the ulcer. In each case, the edge that was perpendicular to the base developed an inclination with epithelialization of the base and contraction of the crater, with eventual complete regrowth of overlying skin.

DISCUSSION

Adriamycin is known to complex with DNA (3,5). This complex has been shown to undergo endocytosis when released from dead cells and in this way to gain further entrance into viable cells, thereby perpetuating the cytotoxic effect.

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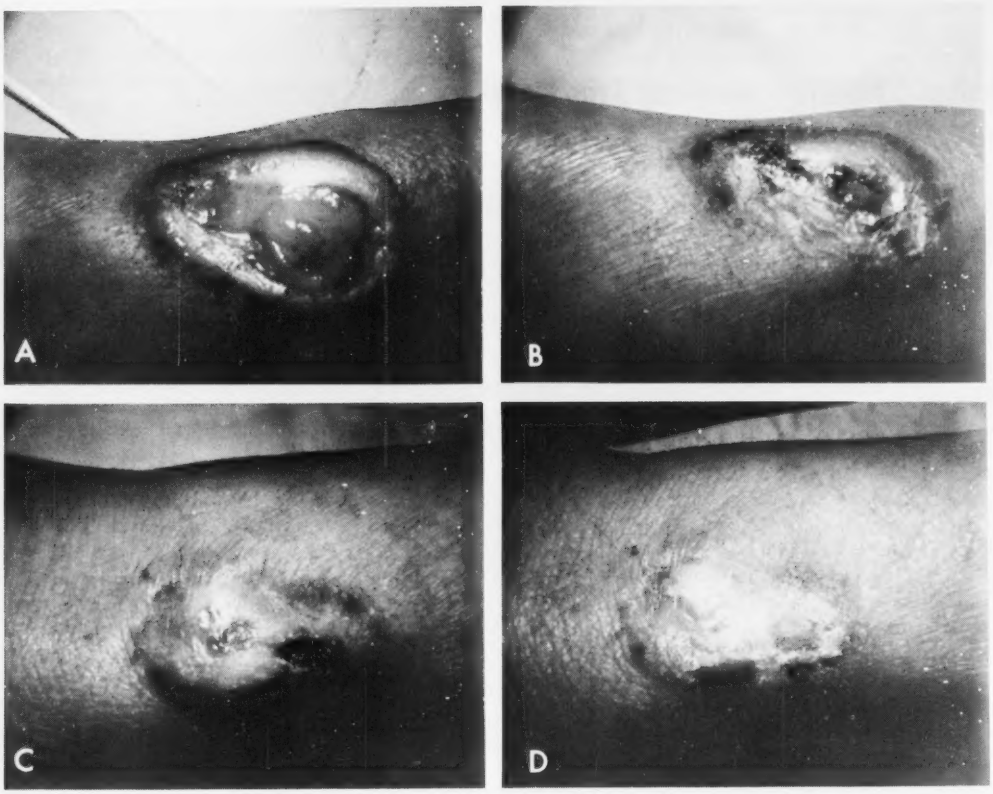

FIGURE 1.—Sequential healing of the Adriamycin ulcer over a 6-wk period; photograph A was taken before therapy.

Sodium hypochlorite has a number of properties that make it ideal for the treatment of the Adriamycin ulcer based upon the picture thus far developed. It is known to dissolve necrotic tissue. During the First World War it was used as a chemical method to clean infected gunshot wounds. It depolymerizes DNA. In the laboratory it has been used to reduce the viscosity of viscous purulent material. It is also an oxidizing agent, releasing free chlorine. This compound degrades highly conjugated organic molecules such as Adriamycin. With these considerations as a basis, we thought it reasonable to expect sodium hypochlorite solution to be a useful substance for destroying the presumptive Adriamycin-DNA complex and inhibiting the local Adriamycin effect. Macerated devitalized skin can be mistaken for the base of the Adriamycin ulcer. If present, it may function as a barrier preventing permeation of the ulcer crater with the sodium hypochlorite solution. Therefore, the ulcer should be free of necrotic tissue.

The use of dextranomer for the treatment of the Adriamycin ulcer was based upon the following considerations. Polymers of this type are hydrophilic substances that have a strong inclination to undergo hydrogen bonding with a great variety of substances. Similar polymers, such as Sephadex, are used in the laboratory to remove numerous organic molecules from solution. In the clinical setting, the hydrophilic colloidal nature of the polymer results in the removal of fluid, with hydrogen bonding of the soluble constituents (6,7). Concomitantly, there would be entrapment of the colloidal components between polymer particles. Because of these characteristics we believed that any of the Adriamycin-DNA complex which had not undergone degradation would be removed by the dextranomer, thereby preventing endocytosis.

An alternate explanation would be that healing occurred spontaneously and was only coincidentally associated with the treatment described. In view of the nature of the Adriamycin ulcer, this explanation is highly unlikely.

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Phase II Evaluation of Chlorozotocin in Advanced Bronchogenic Carcinoma [1,2]

Brief Reports and Preliminary Communications

Edward T. Creagan,* Robert T. Eagan, Thomas R. Fleming, Stephen Frytak, Larry K. Kvols, and James N. Ingle [3]

Chlorozotocin, a 2-chloroethyl-substituted nitrosourea, has antitumor activity against L1210 and P388 leukemias and B16 malignant melanoma with some long-term survivors seen in each tumor system.[4] At the maximally effective dose of chlorozotocin significant myelosuppression was not observed against L1210 leukemia, whereas comparable doses of BCNU did inhibit bone marrow DNA synthesis (1). A phase I study of chlorozotocin identified the dose-limiting toxic effects as leukopenia and thrombocytopenia at doses of 150–200 mg/m^2/day every 6 weeks (2). Another phase I assessment reported thrombocytopenia but not leukopenia to be dose-limiting and recommended a phase II dose of 120 mg/m^2/day every 6 weeks (3). There was no significant cumulative myelosuppression in most patients in either study. The occurrence of objective regression in bronchogenic adenocarcinoma (3), clinically tolerable nausea and vomiting, and a single-day schedule made chlorozotocin an attractive agent for phase II studies of patients with advanced lung cancer. In addition, there is evidence of antitumor effectiveness without myelotoxicity (3).

MATERIALS AND METHODS

Patients were considered eligible if they had histologic or cytologic evidence of measurable or evaluable metastatic adenocarcinoma or large cell carcinoma of the lung.

Thirty-one patients were entered in the study and all were included in the analysis. Twenty-three patients had received prior chemotherapy primarily with combinations including cyclophosphamide, Adriamycin, and *cis*-dichlorodiammineplatinum(II). Thirty-two percent of patients had Eastern Cooperative Oncology Group (ECOG) performance scores of 0 or 1 and 68% had ECOG performance scores of 2 or 3. Patients with a performance score of 4 were excluded from the study. The pretreatment patient characteristics are shown in table 1.

Chlorozotocin was administered by rapid iv infusion at a dose of 125 mg/m^2 every 5 weeks. Patients were removed from the study at the first sign of disease progression.

Patients with measurable or evaluable indicator lesions were evaluated using previously reported response criteria (4). Patients who died or who failed to return for treatment were considered to have tumor progression on the date of death or on the day of scheduled return, whichever occurred earlier. No patients were lost to followup.

RESULTS

Therapeutic results.—There was only one objective response among the 28 patients with adenocarcinoma of the lung: a 60-year-old female who had received no prior chemotherapy had a partial regression for 6 months. There were no regressions among the three patients with large cell carcinoma of the lung. Of the 31 patients, 16 had disease progression during the first 5 weeks after starting treatment and an additional 12 had disease progression during the next 5 weeks, with all patients, except for the single responder, having disease progression by 15 weeks. The 2-, 4-, and 6-month survival probabilities were 71%, 48%, and 20% respectively.

Toxic effects.—Nausea and vomiting were the most frequent side effects, and they were of mild to moderate degree in ten of 31 patients. The one patient who responded received seven cycles of chlorozotocin and developed severe nausea and vomiting and a flu-type syndrome after the last four treatments which necessitated discontinuation of treatment. Five patients had leukopenia (wbcs < 4100/mm^3) with wbc count nadirs of 2300, 3100, 3200, 3400, and 3400/mm^3 occurring on Days 8, 8, 36, 14, and 23 respectively after the start of treatment. Nine patients had thrombocytopenia (platelets < 130,000/mm^3) with a median platelet count nadir of 80,000/mm^3 (range, 22,000–115,000/mm^3) which occurred a median of 21 days after starting chlorozotocin.

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TABLE 1.—Pretreatment characteristics of 31 patients treated with chlorozotocin

Characteristic	No. of patients		
	No prior chemotherapy	Prior chemotherapy	Totals
No. of evaluable patients	8	23	31
Cell type			
Adenocarcinoma	8	20	28
Large cell carcinoma	0	3	3
Age in yrs (median)	(70)	(58)	(58)
Range			
26–49	1	7	8
50–64	1	13	14
65–76	6	3	9
Sex			
Males	4	12	16
Females	4	11	15
ECOG performance score			
0	0	2	2
1	1	7	8
2	6	11	17
3	1	3	4

DISCUSSION

Previous studies of patients with advanced adenocarcinoma of the lung noted response rates of 23% and 35% from methyl-CCNU and CCNU respectively (5). For large cell cancer, CCNU produced objective regression in 17% of patients (5), and one study reported a long-term survivor (4 years) following single-agent treatment with a nitrosourea (6). However, in an earlier study we showed no benefit from either of those agents among 47 patients with adenocarcinoma of the lung, only one responder to methyl-CCNU among 12 patients with large cell carcinoma of the lung, and no responders to CCNU among 13 other patients (7). In our current study, we were again unable to demonstrate any substantive benefit from another nitrosourea (chlorozotocin), even in patients with no prior chemotherapy and relatively good performance scores. We recognize that only eight patients in our study had had no prior chemotherapy and that only 14 of 28 patients with adenocarcinoma demonstrated any myelosuppression. It seems doubtful that higher response rates would be achieved by increasing the dose to a level consistently producing myelosuppression since a previous report noted antitumor activity in the absence of myelosuppression (3). We can reject, at the $\alpha = 0.05$ level of significance, the hypothesis that the objective tumor response rate is at least 15% in similar patients with drug-refractory advanced bronchogenic carcinoma of the adenocarcinoma type.

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Phase II Study of High-Dose *cis*-Dichlorodiammineplatinum(II) in the Treatment of Non-Small Cell Lung Cancer[1,2,3]

Brief Reports and Preliminary Communications

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cis-Dichlorodiammineplatinum(II) (DDP) is an antitumor agent with activity in a broad spectrum of human malignancies (1,2). Objective responses were seen in early clinical trials in non-small cell lung cancer (NSCLC) using DDP in a variety of dose schedules (3–7). The primary dose-limiting toxicity is renal, but the therapeutic index may be improved by mannitol-induced diuresis permitting the administration of higher doses of this drug (7). Although DDP-containing combinations have demonstrated frequent responses in NSCLC (8–11), a phase II trial of high-dose DDP (120 mg/m^2) as a single agent has not been previously reported.

MATERIALS AND METHODS

Nineteen patients were entered in a phase II study of high-dose DDP in NSCLC. All patients had objectively measurable disease and histologic or cytologic confirmation of the diagnosis of NSCLC. A Karnofsky scale performance status (PS) of ≥ 50%, a wbc count of ≥ 4000/mm^3, a platelet count of ≥ 100,000/mm^3, and a creatinine clearance of ≥ 60 ml/minute were required for entry in this study. Eighteen patients were evaluable for therapeutic response. The median age of the patients was 55 years (range, 33–70), and the median PS was 70% (range, 50%–80%) on the Karnofsky scale. Ten patients had not received any prior chemotherapy. The histologic types were adenocarcinoma (nine patients), epidermoid carcinoma (six patients), and large cell carcinoma (three patients).

The method of hydration, diuresis, and DDP administration has been previously described (7). Briefly, during the 12–18 hours before DDP therapy, 2–3 liters of 5% dextrose in 0.45% NaCl was administered iv to all patients. Immediately before therapy, mannitol (12.5 g) was given as a rapid iv injection. DDP (3 mg/kg or 120 mg/m^2) was then administered by rapid iv infusion over approximately 15 minutes. For the next 6 hours, patients received constant iv infusion of 20% mannitol at a dose of 50 ml/hour and 5% dextrose in 0.45% NaCl at a dose of 200 ml/hour. After the first 6 hours, the total fluid output was replaced with an equal volume of 5% dextrose in 0.45 NaCl with KCl (20 mEq/liter) every 2 hours for at least 18 hours. Doses were repeated at 4–6-week intervals in the absence of disease progression if the creatinine clearance remained > 60 ml/minute.

Therapeutic responses were defined as follows: *complete remission*—the disappearance of all evidence of tumor for at least 4 weeks; *partial remission (PR)*—a ≥ 50% reduction of the sum of the products of the two greatest perpendicular diameters of all measurable lesions for at least 4 weeks; and *minor response*—a > 25% reduction of measurable lesions, but less than that required for a PR. Patients were considered adequate for evaluation after 8 weeks and two doses of DDP or 4 weeks after a single dose of the drug in the presence of unequivocal evidence of progressive disease.

RESULTS

Therapeutic Responses

Eighteen patients were evaluable for therapeutic response; an additional patient died of progressive disease 2 weeks after initiation of therapy and is not considered adequate for evaluation. One PR was noted among the 18 evaluable patients; it occurred in a previously untreated male with epidermoid carcinoma and a PS of 70%. The patient's pulmonary mass regressed for 5 months.

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[3]*cis*-Dichlorodiammineplatinum(II) was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

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[5]We wish to thank Mary Dukeman for assistance with analysis of the data and Polly Hodge and Carol Turner for preparation of the manuscript.

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TABLE 1.—Toxicity of high-dose DDP in 18 patients with NSCLC

Toxic effect	No. of patients
Wbc count nadir (cells/mm³)	
Median: 6100	
Range: 2600–11,800	
2500–3900	3
≥4000	15
Platelet count nadir (cells/mm³)	
Median: 207,000	
Range: 36,000–574,000	
<100,000	1
100,000–200,000	4
>200,000	13
Hemoglobin decrease (g/dl)	
Median: 1.8	
Range: 0.2–3.2	
<1.0	4
1.0–2.0	10
>2.0	4
Peak serum creatinine (mg/dl)	
Median: 1.4	
Range: 0.9–2.9	
<1.0	2
1.0–1.9	13
2.0–3.0	3

Two minor responses were also noted; both of these patients had epidermoid carcinoma and a PS of 80% and had received prior chemotherapy. One of these patients experienced transient reduction of pulmonary nodules after each of four doses of DDP with slight regrowth of the nodules between each dose. The other patient had prompt reduction of the pulmonary mass after his first dose; this minor response lasted for 7 months. Four of the remaining 15 patients had stable disease for 2–4 months and were subsequently treated with other agents. The other 11 patients had disease progression; three had only one dose of DDP and the other eight had two doses.

Toxicity

A median of two doses (range, one to six) were administered to each patient. Hematologic and renal toxicity are outlined in table 1. Myelosuppression was mild and there was no incidence of drug-related sepsis or hemorrhage. Only three patients developed a peak serum creatinine of > 2.0 mg/dl (normal: < 1.4 mg/dl). Although progressive mild deterioration of creatinine clearance was noted in four patients who received more than two doses of DDP, no patient required treatment for renal failure. Tinnitus and transient worsening of hearing were common, but no patient suffered any permanent clinically significant hearing loss. Nausea and vomiting were generally moderate to marked in severity and occurred in all patients in this study.

DISCUSSION

DDP is active in a variety of malignant neoplasms (1,2). Several investigators have combined DDP with other agents for the treatment of NSCLC and have reported major response rates in the range of 18%–65% (8–11). The role of DDP as a single agent in the treatment of this disease, however, has been unclear. In reviewing early clinical trials of DDP used in a variety of doses and schedules, three objective responses were noted among 40 patients (8%) with NSCLC (3–6). With only one PR occurring among 18 evaluable patients, the results of our study do not support a superior response rate for the high-dose regimen.

Two recent phase II trials of DDP in NSCLC included patients with good PS who had received no prior chemotherapy. Britell et al (12) reported two PRs among 13 previously untreated patients with measurable disease. Rossof et al (13) noted one PR in a previously untreated patient; six patients in that study had had no prior chemotherapy.[6] In the present report, the PR was observed in one of the ten patients who had received no prior chemotherapy. Therefore, in the combined experience of these three studies, the major objective response rate is 14% (four of 29 patients) in patients who had received no prior chemotherapy. At 95% confidence limits, the predicted true response rate is in the range of 5%–30%. Although this is not as high a response rate as that seen with DDP in the treatment of other tumors, this activity is in the range of that reported for older single agents currently used in the treatment of NSCLC (14,15). In light of its activity with only minimal myelotoxicity, further investigations of DDP in combination with other active agents in previously untreated patients with this disease appear to be indicated.

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Preliminary Report on the Clinical Use of Human Leukocyte Interferon in Neuroblastoma [1,2]

Brief Reports and Preliminary Communications

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SUMMARY

The effectiveness of human leukocyte interferon (IF) injections on a female patient, 21 years old, with stage IV neuroblastoma was observed. IF (30×10^4 units) injected every second day intratumorally or around the tumor tissue rapidly reduced the tumor size. However, systemic administration of 300×10^4 units of IF by im injection showed no beneficial effects. Our results suggest that a high IF level in tumor tissue may be necessary to bring about tumor reduction.

[Cancer Treat Rep 63:2111–2113, 1979]

In 1974, Strander et al (1) suggested that human leukocyte interferon (IF) may have some beneficial effects in patients with osteogenic sarcoma, such as prophylaxis of pulmonary metastases and prolongation of the survival period. IF was experimentally administered to patients with malignant diseases, and the results were evaluated (2,3).

This study evaluates the effectiveness of local and systemic administration of human leukocyte IF to a patient with widespread neuroblastoma.

MATERIALS AND METHODS

The patient was a 21½-year-old female with widespread neuroblastoma which was estimated as stage IV (4). At 16 years of age, she was diagnosed as having an abdominal neuroblastoma by histologic examination of biopsied specimens taken at the time of her first operation. During the 4 years and 6 months since the initial diagnosis, she received various treatments prior to IF therapy as follows: four operations, Co^{60} irradiation (total, 7800 rads), cyclophosphamide (15,750 mg iv and 67,875 mg orally), vincristine (158.7 mg), daunorubicin (86.5 mg), Adriamycin (180 mg), and papaverin (12,000 mg).

In May 1978, when the patient was 21 years old, two new masses measuring 2–3 cm in diameter were detected in the left neck, and urinary vanillylmandelic acid (VMA) and homovanillic acid (HVA) showed higher levels (VMA = 39.2 and HVA = 45.9 μg/mg of creatinine). Her metastatic neck masses increased in number and size as shown in figure 1. After giving informed consent, she was admitted again for IF therapy for metastatic masses.

Human leukocyte IF was prepared at the Kyoto 1st Red Cross Hospital and the Green Cross Corp in Japan. Vials containing either 30×10^4 or 300×10^4 IU were prepared. IF was dissolved in a 0.5% human plasma solution at the time of injection. These preparations did not contain endotoxin, demonstrated by either human skin test or limulus assay.

Tumor A was injected with 30×10^4 units of IF intratumorally; for tumor B the same amount was injected around the mass, and for tumor F a 0.5% human plasma solution was administered intratumorally every second day (fig 1). After ten local injections, 300×10^4 units of IF was administered six times im every second day. The total IF dose was 2400×10^4 units.

During this study, all superficial metastatic masses detected by palpation were measured serially, and sizes were recorded by multiplying the greatest diameters by the shortest diameters (fig 1). By calculating the changes in the tumors, the effect of IF injection was evaluated.

RESULTS

Serial changes in tumor size are shown in figure 1. A reduction in tumor A was observed within 1 week but for tumor B reduction was perceptible only gradually. Tumors C and D, which were subsequently discovered (at autopsy) to be parts of tumor A, were reduced in the same manner as tumor A during local administration of

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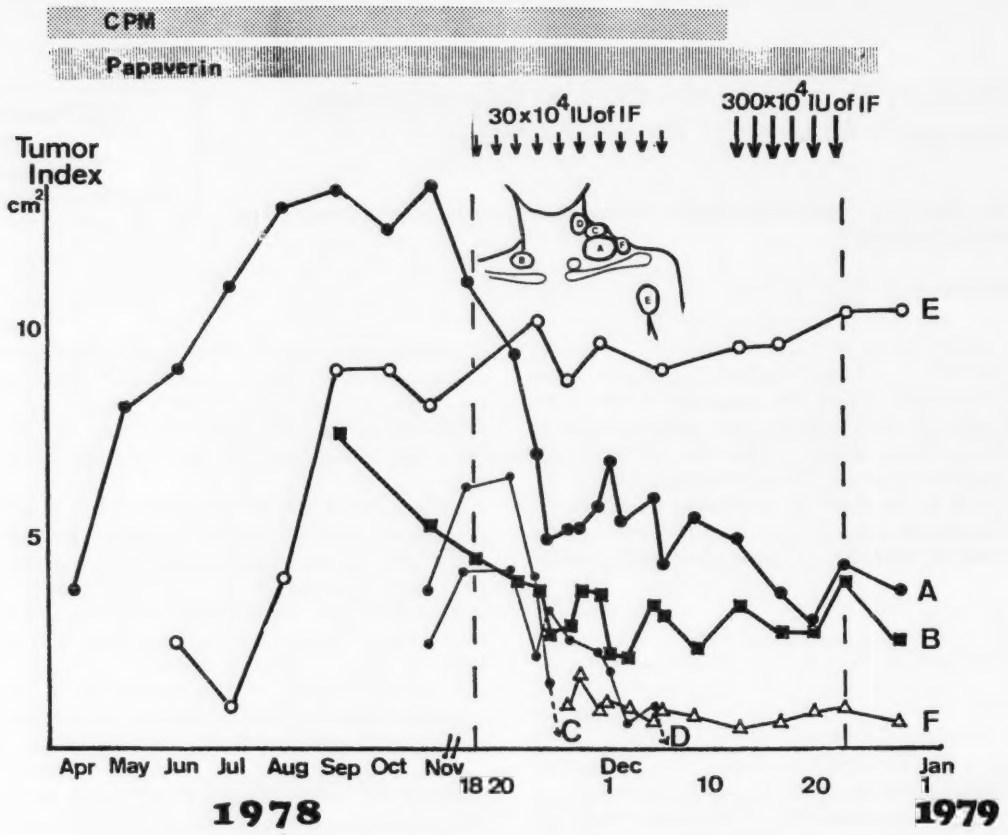

FIGURE 1.—Results of IF administration in a 21-yr-old female with neuroblastoma.

IF to tumors A and B, and they disappeared within 3 weeks after beginning local IF injections. Tumor E, located at the left axilla, and tumor F, injected with a 0.5% human plasma solution, did not decrease in size.

One week after the cessation of the local administration of IF, systemic administration of 300 × 10^4 units of IF was started by im injection. Simultaneously, cyclophosphamide therapy had to be discontinued due to increasingly worse hemorrhagic cystitis. This systemic administration did not show any beneficial effect on tumor reduction. By the 9th day following the cessation of IF administration, the tumor had not changed in size. The patient died of severe cachexia on the 14th day.

There were no other side effects except for shivering and high fever which returned to normal within 1 hour following severe sweating. There were no changes in blood chemistries (LDH, SGOT, SGPT, etc) or in immunologic data (serum C3, C4, CH50, and immunoglobulin, and skin reactions of purified protein derivative and phytohemagglutinin) during and after IF administration. Effects on peripheral blood cell counts were not evaluated because blood transfusions were performed frequently. Urinary VMA and HVA levels increased to 101.2 and 309.2 μg/mg of creatinine before IF therapy, but during and after IF therapy they were not measured due to macrohematuria.

DISCUSSION

This study was the first clinical trial of human leukocyte IF administration to a patient with neuroblastoma. It was observed that local IF administration was effective in reducing tumor size. Although the effect of systemic administration could not be estimated, none of the masses increased in size. If a larger amount of IF had been administered, it would have produced more beneficial results. The 0.5% human plasma solution alone showed no effect even with intratumoral injection. These results may suggest that IF is an effective substance for the treatment of progressive neuroblastoma, although the mechanism of reducing the tumor cells is unknown.

Investigations of intratumoral IF injections in cancer patients have been done previously. In 1977, Horoszewicz et al as reported by Leff (5) injected 50×10^4 units of IF into melanomas every day for several weeks. In 1978, Habif et al (personal communication) injected 320×10^4 units of IF into mammary carcinomas two times daily for a 12-day period. Results of both investigations showed the beneficial effects of IF in reducing or eradicating these tumors.

Additional studies will be needed before the usefulness of IF for neuroblastoma is known.

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LETTERS

Nephrotoxic Potential of the Cephalosporin-Aminoglycoside Combination

An Alternate View

I read with interest the article "Acute Renal Failure After *cis*-Dichlorodiammineplatinum(II) and Gentamicin-Cephalothin Therapies" by Gonzalez-Vitale et al in the May 1978 issue of *Cancer Treatment Reports*. The clinical situation described in the article is assuming increasing importance in the management of various malignancies with regimens which include *cis*-dichlorodiammineplatinum(II) (cisplatin). From the data given, however, I am not certain that a true potentiation in renal failure was demonstrated with the addition of gentamicin-cephalothin to cisplatin therapy. Little clinical information on these patients was included in the article. Therefore, one cannot judge if there were clinical circumstances predisposing to the development of cisplatin-induced renal failure, apart from the addition of nephrotoxic antibiotics. The description of the histopathologic features of the kidneys in the cases presented adds nothing to the authors' case, since the changes described are all nonspecific. No electron-microscopic findings were included in the study which might show evidence of tubular damage associated with gentamicin. The time course of elevation in the serum creatinine levels depicted in the figures is not incompatible with renal toxicity secondary to cisplatin alone.

With the increasing use of cisplatin in combination with myelotoxic agents, the incidence of chemotherapy-related febrile episodes will increase. The aminoglycoside antibiotics, particularly when used in combination with a cephalosporin, have been extremely useful in the management of this complication of cancer chemotherapy. If, indeed, caution needs to be used in administering these drugs after cisplatin therapy, then stronger evidence should be available. Otherwise, withholding these antibiotics might lead to suboptimal management of infectious complications in these patients.

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Author's Response

In our paper we did not claim to have demonstrated a "true potentiation" of cisplatin nephrotoxicity with gentamicin-cephalothin therapy. The data reported showed that four patients treated with cisplatin and gentamicin-cephalothin died with severe acute renal failure. This outcome was unlike that of those patients treated only with high-dose cisplatin plus mannitol diuresis, who developed mild transient elevation of serum creatinine levels (1).

The possibility that this severe acute renal failure was due to cisplatin toxicity alone, as suggested by Dr. Liepman, is unlikely, based on the fact that following the cisplatin therapy, the azotemia was mild and transient in three patients and was steadily abating in the fourth, who had severe renal failure. These facts were in evidence before administration of the antibiotic combination.

As stated in the Materials and Methods section, our patients did not have any underlying renal disease and had not received previous treatment with nephrotoxic drugs when the cisplatin was started. Furthermore, after the cisplatin therapy, none of the patients were in circulatory shock, which is another condition that can induce acute tubular necrosis. We were not, therefore, aware of "clinical circumstances" in our patients that could predispose, potentiate, or add to the cisplatin nephrotoxicity, other than the known potentially nephrotoxic combination of gentamicin and cephalothin.

Contrary to Dr. Liepman's opinion, the gross and microscopic pathologic findings in our patients actually did show that the patients died with severe acute tubular necrosis. The microscopic lesions were qualitatively similar to and quantitatively much more extensive than the lesions seen in patients treated with high-dose cisplatin and mannitol diuresis alone (2). Needless to say, we did not claim that these changes were specific.

Whatever the pathogenesis might be of the severe acute renal tubular necrosis seen in our patients after cisplatin and gentamicin-cephalothin therapies, the fact remains that it was a major complication of treatment.

There is now increasing evidence that the combination of an aminoglycoside antibiotic and cephalothin may be substantially nephrotoxic per se, let alone in those patients who also receive cisplatin.

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November 20, 1979

Editorial Response

The prompt empirical use of broad-spectrum, bactericidal antibiotics in granulocytopenic cancer patients

who have developed fever is a critical, effective technique to prevent rapid progression of infection and death. Antibiotic combinations encompassing a wide spectrum of organisms, principally gram-negative bacilli, which cause infection in these patients are indicated. The most popular combinations with proven efficacy include the three possible two-drug combinations of cephalothin, carbenicillin, and gentamicin (or their analogs such as cefazolin, ticarcillin, tobramycin, and amikacin) or the three drugs used together.

During recent years reports have suggested that the combination of cephalothin and gentamicin is more nephrotoxic than either drug used alone. Recently, three large prospective controlled evaluations have conclusively shown that there is an increased nephrotoxic potential when cephalothin is used in combination with either gentamicin or tobramycin. Klastersky et al (1) compared the use of cephalothin-tobramycin to cephalothin-ticarcillin and to ticarcillin-tobramycin in 186 cancer patients treated for suspected sepsis. Antibiotic-related nephrotoxicity (rise of creatinine to $>$ 2 mg/100 ml) was found in 21%, 2%, and 6% of the patients respectively ($P < 0.05$). The Antimicrobial Therapy Project Group of the European Organization for Research on Treatment of Cancer (EORTC) (2) evaluated 625 febrile episodes in granulocytopenic cancer patients treated with cephalothin-gentamicin, cephalothin-carbenicillin (or ticarcillin), or carbenicillin (or ticarcillin)-gentamicin. Serial creatinine levels were available for 365 patients whose initial creatinine level was $\leq$ 1.5 mg/100 ml. Because of the findings of Klastersky et al, the EORTC group reviewed the data for all patients who experienced a creatinine rise to a level $>$ 1.5 mg/100 ml to ascertain whether the rise was secondary to the antibiotics or to another potential cause of renal damage (eg, hypotension, other drugs, etc). They found that severe antibiotic-related nephrotoxicity (creatinine rise to a level $\geq$ 2.5 mg/100 ml from a level of $\leq$ 1.5 mg/100 ml) developed in 12%, 4%, and 2% of the patients in the three groups respectively, with significantly ($P < 0.05$) more nephrotoxicity occurring in the cephalothin-gentamicin-treated patients. When the incidence of nephrotoxicity was adjusted for age, it was found that the cephalothin-gentamicin combination induced severe renal damage in 27% of those patients $>$ 60 years of age compared to no cases of renal damage for the other two combinations. Similarly, among those patients with initial high-normal creatinine levels (1.0–1.4 mg/100 ml), severe nephrotoxicity developed in 21% of the cephalothin-gentamicin group compared to 7% for the other two groups ($P < 0.05$). Thus, older patients with initial high-normal creatinine levels were most likely to develop nephrotoxicity. Wade et al (3) compared four regimens in a double-blind prospective trial: cephalothin with either gentamicin or tobramycin and methicillin with either gentamicin or tobramycin given to 135 patients for suspected gram-negative sepsis in a general hospital setting. Antibiotic-related nephrotoxicity was defined as a rise in creatinine of $>$ 0.4 mg/100 ml without other apparent cause. The aminoglycoside-cephalothin regimens resulted in significantly more nephrotoxicity (26%) than the aminoglycoside-methicillin combinations (7%; $P = 0.017$).

At the National Cancer Institute (Baltimore Cancer Research Program), we have recently reviewed all 698 patients treated with empirical antibiotic combinations over a 5-year period (4). Combinations included those of the previously described EORTC trial, cephalothin-amikacin, ticarcillin-amikacin (or netilmicin or gentamicin), and ticarcillin-amikacin with or without cefazolin. There were 491 patients evaluable based upon the criteria of $\geq$ 3 days of therapy plus baseline and followup creatinine values. The cephalothin-aminoglycoside (gentamicin or amikacin) regimens were significantly more nephrotoxic (18%) than the carbenicillin (ticarcillin)-aminoglycoside regimens (3%; $P < 0.001$). This increase in nephrotoxicity was augmented by increasing age. Of interest, the ticarcillin-cefazolin-amikacin regimen was not notably nephrotoxic (6%), consistent with the results of the current EORTC trial (comparing carbenicillin [ticarcillin]-amikacin with or without cefazolin) which has now enrolled 711 patients without evidence of excess nephrotoxicity (5). It should be noted that at least one large retrospective evaluation of the cephalothin-aminoglycoside regimen has not noted major degrees of nephrotoxicity (6). It has been suggested that the three-drug combination of carbenicillin-cephalothin-gentamicin is substantially nephrotoxic (7), although, as noted above, the EORTC experience with the carbenicillin (ticarcillin)-cefazolin-amikacin combination appears safe.

The results and implications of these evaluations are clear. The combination of cephalothin plus an aminoglycoside (gentamicin, tobramycin, or amikacin) is substantially nephrotoxic. It would therefore be prudent to avoid these combinations whenever possible, especially in the patient who is older or the patient who has received other potentially nephrotoxic agents such as cisplatin. However, cephalothin plus an aminoglycoside remains the therapy of choice for *Klebsiella pneumoniae* infections and is probably the best regimen in other gram-negative infections where the two agents are synergistically active against the offending pathogen. Potential nephrotoxicity should not discourage the use of cephalothin plus an aminoglycoside for these select infections. In other situations, initial therapy with ticarcillin or carbenicillin plus either an aminoglycoside or a cephalosporin would seem more appropriate unless there was strong epidemiologic evidence (eg, hospital infection patterns, results of recent patient surveillance cultures, etc) to suggest the use of cephalothin plus an aminoglycoside. While a potentiation of antibiotic-associated nephrotoxicity by cisplatin may not have been established with certainty

(8), the likelihood is sufficiently great that extreme caution should be utilized in the choice of antimicrobial therapy following the use of this antitumor agent.

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An Observation on the Stability of *cis*-Dichlorodiammineplatinum(II): A Caution Regarding Its Administration

There has recently been a great deal of interest and research in the area of *cis*-dichlorodiammineplatinum(II) (cisplatin) stability in aqueous solutions (1–3). The stability of this complex has been studied in aqueous solutions with respect to varying concentration, sodium chloride content, and the presence or absence of 5% dextrose, 5% mannitol, and 5% sodium bicarbonate. We would like to report on our observations concerning the instability of cisplatin during administration of the drug to a patient at Women's Hospital, Long Beach, CA.

The commercially available cisplatin preparation was reconstituted with sterile water for injection to yield a solution containing 1 mg/ml. This solution was then administered to the patient according to the procedure specified in the Gynecologic Oncology Group (GOG) protocol 26C, whereby the solution containing 1 mg of cisplatin/ml was piggybacked into a patent iv line (5% dextrose in lactated Ringer's solution) and infused at the rate of 1 mg/minute. This method utilized an Abbott 150-ml sterile evacuated container which held the solution and an Abbott secondary piggyback tubing set No. 1832 which delivered the solution through a Monoject 250 19G 1-inch needle into the primary iv line. By the end of the infusion (55 minutes), a brownish precipitate was found within the secondary piggyback tubing. The precipitate was confined to the secondary piggyback tubing and accumulated primarily about the inner hub of the needle of the secondary tubing set. This inner hub holds the needle shaft, and in turn is held by a plastic casing which forms a female adapter to fit the male adapter at the end of the tubing. The inner hub is composed of aluminum and is in contact with the cisplatin solution throughout its administration.

This observation prompted us to test the reactivity of a 1-mg/ml cisplatin solution when in physical contact with various metals. We prepared a solution identical to that which was used for administration to the patient and placed 5-ml aliquots into three separate test tubes; samples of elemental aluminum (same substance as the hub of the Monoject needle), stainless steel (same substance as the needle shaft), and copper (used for test purposes only) were placed into each tube respectively. After a few minutes, a brownish precipitate began forming on the surface of the aluminum, but not on the surface of the other two metals. At the end of 1 hour, the tube containing the aluminum had accumulated a substantial amount of this brownish precipitate while the other tubes had no evidence of reaction. We postulate that the reaction occurring between the elemental aluminum and the cisplatin solution is a replacement oxidation-reduction reaction as follows:

$$2Al^{0} + 3Pt^{++} = 2Al^{+++} + 3Pt^{0}$$

The stainless steel and copper failed to produce a similar reaction with the cisplatin solution which might be explained in several ways: (*a*) The replacement oxidation-reduction reaction between aluminum and platinum has a high-voltage potential, about +2.9 volts (4). High-voltage potentials tend to make reactions take place spontaneously. The corresponding reactions involving copper or iron (stainless steel) have much lower potentials and therefore have less chance of forming a spontaneous reaction. (*b*) The reactivity of the stainless steel is very low because the iron content is stabilized by the addition of chromium, nickel, and other substances to the alloy. (*c*) The platinum specie available in cisplatin is stabilized partially by its complexation with chloride and ammonia.

This could mean that the net voltage potential is reduced so that only aluminum, the most reactive of the three substances tested, takes part in a spontaneous reaction. (*d*) Replacement reactions for stainless steel and copper might have been occurring, but at a rate that was too slow to detect grossly.

In summary, our observation indicates that a 1-mg/ml cisplatin solution is unstable when placed in physical contact with certain metals, especially aluminum. The amount of drug received by the patient is therefore reduced by an unknown amount. The product information supplied by the manufacturer[1] makes no reference to any such instability. Further studies are currently in progress to obtain more information about the stability of cisplatin at various concentrations and in commonly used solutions when in physical contact with metals during drug administration. Until the results of these studies are known, it should be emphasized that cisplatin solutions should not come into contact with any aluminum.

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[1]Package insert for Platinol. Syracuse, NY, Bristol Laboratories, Sept 27, 1978.

Incompatibility of Aluminum-Containing Iv Administration Equipment With *cis*-Dichlorodiammineplatinum(II) Administration

As a result of the preceding report by Bohart and Ogawa documenting incompatibility of a 1-mg/ml aqueous solution of *cis*-dichlorodiammineplatinum(II) (cisplatin) with aluminum metal, Bristol Laboratories has conducted several studies to investigate the interaction of cisplatin with aluminum components of various types of iv administration equipment. Initial experiments included placing metallic aluminum in a 1-mg/ml solution of cisplatin in distilled water at room temperature. Microscopic examination revealed a black precipitate which formed on the aluminum surface within 5–10 minutes after initial contact, accompanied by evolution of gas bubbles. The black precipitate became visible to the unaided eye within 30–60 minutes. Elemental analysis of the black precipitate showed the presence of platinum, aluminum, and oxygen.

Various types of marketed iv administration equipment were subsequently placed in contact with a 1-mg/ml solution of cisplatin and observed over a period of time for formation of a black precipitate. A total of 29 sample units from eight manufacturers of iv administration sets, needles, and indwelling iv catheters were tested in this study. Only the interior surfaces normally exposed to iv solutions were in contact with the test solutions of cisplatin (1 mg/ml, pH 4.5). Observations were made with the aid of a 6 × magnification microscope and with the unaided eye at specific intervals up to 72 hours. A total of six units from three manufacturers were found to be incompatible with cisplatin. Four of these units were aluminum-hubbed needles and two were iv administration sets equipped with aluminum-hubbed needles. Visible evidence of a black precipitate on the aluminum hubs of needles was first detected 1.0 or 1.5 hours after initial contact and became grossly apparent by 24 hours. The only metal parts that were found to be reactive with cisplatin were the aluminum hubs of needles and no other metal incompatibilities were observed with cisplatin in this study.

Experiments were also performed to assess the loss of cisplatin from solutions which were in contact with aluminum-containing disposable needles. Twenty-six milliliters of a 1-mg/ml cisplatin solution in water was transferred to a 125-ml Erlenmeyer flask containing one disposable needle. The flasks were covered with parafilm, kept in the dark at room temperature, and occasionally swirled. At 0.25, 0.5, 1.0, 3.0, and 6.0 hourly intervals the samples were observed for the presence of a black precipitate and aliquots were taken for cisplatin analysis by high-pressure liquid chromatography. A black precipitate was observed at 0.25 hour after initial contact and a 28%–29% loss in cisplatin concentration was detected by high-pressure liquid chromatography at 3 hours. At 6 hours after initiation of the experiment, a 46%–50% loss of cisplatin from the solution had occurred.

The results presented in this report confirm the incompatibility of cisplatin and aluminum reported previously and provide chemical evidence that a replacement oxidation-reduction reaction occurs between aluminum and

the platinum in cisplatin solutions when these two compounds are in contact. Further evidence is presented which identifies the aluminum components of disposable needles and the needles in iv administration sets as the sites of reaction with cisplatin. Up to 50% of the cisplatin is degraded in 6 hours when in contact with an aluminum-hubbed disposable needle. These results strongly emphasize the need to identify aluminum-containing iv administration equipment which should not be used to administer cisplatin-containing solutions to patients.

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Combination Chemotherapy With Cyclophosphamide Plus 5-Fluorouracil for the Treatment of Unresectable Pancreatic Adenocarcinoma[1,2]

Twenty-four patients with unresectable adenocarcinoma of the pancreas were treated with a combination of 5-fluorouracil (5-FU) and cyclophosphamide (CYC). Thirteen patients who received the two highest dose levels (high-dose 5-FU and CYC) had a longer median survival than 11 patients who received the low-dose levels (8.9 versus 3.5 months, respectively; $P < 0.05$). The beneficial effect of the high-dose treatment was seen only in patients without liver metastases. In such patients, median survival was 13.4 months.

The prognosis for unresectable adenocarcinoma of the pancreas is poor. Chemotherapeutic agents are generally ineffective in prolonging survival (1). 5-FU has some therapeutic activity in this disease. The reported cumulative objective response frequency is approximately 20% (1). Experience with alkylating agents in pancreatic carcinoma has been limited, but objective responses to CYC have been reported (2). The purpose of this study was to evaluate whether four dose levels of CYC used in combination with 5-FU in a nonrandomized trial were tolerable and could affect survival.

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[2]The following members and institutions of the Western Cancer Study Group participated in this study: *R. Chlebowski, C. Gota, J. Weiner,* and *J. R. Bateman,* Los Angeles County-University of Southern California Medical Center; *A. Glass,* Kaiser Foundation Hospital, Portland, OR; *F. E. Bull,* University Hospital, Ann Arbor, MI; *T. Godfrey,* Loma Linda University Medical Center, CA; *R. A. Bohannon,* San Francisco, CA; *J. D. McCracken,* Madigan Army Medical Center, Tacoma, WA; *R. W. Phillips,* Veterans' Hospital, Spokane, WA; *J. C. Rosentiel,* Veterans' Hospital, Fresno, CA; *I. Silverberg,* Mt. Zion Hospital, San Francisco, CA; and *M. MacKenzie,* University of California at Davis, Sacramento.

MATERIALS AND METHODS

Twenty-six patients with a diagnosis of biopsy-proven unresectable adenocarcinoma of the pancreas were entered in this study. Other patient-eligibility factors included (*a*) a wbc count of $> 5000/mm^3$ and a platelet count of $> 150{,}000/mm^3$; (*b*) no previous chemotherapy or radiotherapy; (*c*) no evidence of infection or severe renal (creatinine < 2 mg/100 ml) or liver (bilirubin < 5 mg/100 ml) dysfunction; and (*d*) an interval of at least 2 weeks following any major surgical procedure.

Pretreatment studies included physical examination, cbc, biochemical studies, chest roentgenogram, and liver scan. Partial objective response was defined as a reduction in tumor size of $\geq 50\%$ of the cross-sectional area of all measurable lesions with the appearance of no new lesions for a period of ≥ 1 month. The log-rank test on Kaplan-Meier-type survival curves was used to assess the statistical significance of survival. Four dose levels of 5-FU and CYC were given, with each treatment group receiving only one dose level. When a dose level was nonprohibitive in toxicity it was closed to further patient entry and treatment assignments to the next highest dose level were made. At all dose levels CYC was given iv on Day 1, while 5-FU was given iv weekly on Weeks 2–5 beginning on Day 8. This cycle was repeated at 6-week intervals. Dose levels were as follows: *Level 1*—10 mg/kg of CYC and 12.5 mg/kg of 5-FU; *Level 2*—15 mg/kg of CYC and 12.5 mg/kg of 5-FU; *Level 3*—15 mg/kg of CYC and 15 mg/kg of 5-FU; and *Level 4*—20 mg/kg of CYC and 15 mg/kg of 5-FU. Levels 1 and 2 are considered low-dose treatment and Levels 3 and 4 are considered high-dose treatment.

RESULTS

Twenty-four of the 26 patients received at least one dose of CYC and 5-FU and were considered evaluable for toxicity and response. Pretreatment characteristics of these patients are presented in table 1. Eleven patients in each of the high- and low-dose groups had measurable disease. Two patients in the high-dose group had a partial tumor response and lived for > 1 year. No responses were seen in patients in the low-dose group. Measurable disease most frequently consisted of an abdominal mass in

TABLE 1.—Pretreatment patient characteristics

Characteristic	Treatment	
	Low-dose	High-dose
No. of evaluable patients	11	13
Median age in yrs	64	57
Median performance status (Karnofsky scale)	70	60
% of patients with—		
Liver metastasis	73	46
Surgical procedure		
Bypass	45	46
Biopsy only	55	54
Primary tumor location		
Head	72	69
Body	28	31

patients recovering from laparotomy. Since changes in such parameters may not accurately reflect tumor response, survival data may give the most reliable information regarding treatment effectiveness.

The patients treated with high-dose CYC and 5-FU had a significant increase in survival (median, 8.9 versus 3.5 months; $P < 0.05$) when compared to those patients receiving low-dose treatment (table 2). All eight of the patients who lived for > 8 months received the high-dose combination. Included in table 2 is the survival of 26 patients who met the same diagnostic criteria; these patients were comparable in prognostic variates and were treated with 5-FU alone by the Western Cancer Study Group in an immediately preceding trial (3). The survival of the high-dose CYC and 5-FU patient group also was significantly greater than that of this historical group ($P < 0.05$). The Cox regression model was used to assess the following pretreatment characteristics: sex, age, performance status (Karnofsky scale), Hgb, wbc count, location of primary lesion, extent of metastatic disease, and dose level of chemotherapy. Using this model, liver involvement and chemotherapy level (ie, high- or low-dose) were found to be independent factors having a significant relationship with survival.

Liver metastases were more common in the low-dose group patients than in the high-dose group patients (73% versus 46%, respectively [table 1]). Liver involvement did not appear to influence survival in patients treated with low-dose CYC and 5-FU, since all patients receiving this treatment had short survival times (table 2). A similar result was seen in the previous study using 5-FU alone (3). However, in the high-dose CYC and 5-FU treatment group, patients without liver involvement did much better, demonstrating significantly increased survival compared to those patients with liver metastasis (median, 13.4 versus 2.7 months; $P < 0.05$ [table 2]). Thus, it appears that the beneficial effect of the high-dose CYC and 5-FU combination treatment in pancreatic carcinoma occurs only in patients who are without liver involvement.

Nausea was reported in 54% of patients in the high-dose group and in 27% in the low-dose group. There were no life-threatening hematologic toxic effects. Six patients in the high-dose group had leukopenia (< 2000 cells/mm^3 with none < 1000 cells/mm^3); leukopenia (< 2000 cells/mm^3) was not seen in the low-dose group. One patient in each group had severe thrombocytopenia (< 50,000 cells/mm^3).

DISCUSSION

Until recently, the use of combinations of agents in the treatment of advanced pancreatic cancer has been disappointing, with survival generally not improved from that seen with 5-FU alone (1). Encouraging results have now been obtained with several regimens. A combination of streptozotocin, mitomycin C, and 5-FU has resulted in increased response frequency in disseminated pancreatic cancer in two nonrandomized trials (4,5). Another three-drug combination, 5-FU, Adriamycin, and mitomycin C, produced a 43% objective response rate and a median survival of 6.5 months in a series of 14 patients (6). In the present study, patients treated with high-dose CYC and 5-FU experienced a significant prolongation of survival when compared to those patients treated with low-dose CYC and 5-FU and, retrospectively, to similar patients treated with 5-FU alone (3). These promising regimens (streptozotocin, mitomycin C, and 5-FU; 5-FU,

TABLE 2.—Median survival of patients with pancreatic adenocarcinoma

Treatment	Drug	Survival (mos) of—		
		All patients (No.)	Patients (No.) without liver metastasis	Patients (No.) with liver metastasis
High-dose (Levels 3 and 4)	CYC + 5-FU	8.9 (13)*	13.4 (6)*	4.5 (7)
Low-dose (Levels 1 and 2)	CYC + 5-FU	3.5 (11)	2.7 (3)	3.7 (8)
Historical (ref 3)	5-FU	4.2 (26)	4.4 (12)	4.0 (14)

*Significantly different ($P < 0.05$) from both low-dose CYC + 5-FU and historical 5-FU.

Adriamycin, and mitomycin C; and CYC and 5-FU) all require confirmation in prospective randomized trials.

An analysis of multiple pretreatment variables in these patient groups identified liver involvement as a factor significantly related to patient survival. The importance of liver involvement was best seen when effective chemotherapy was used. Survival was not increased in patients without liver involvement treated with either low-dose CYC and 5-FU in the present study or with 5-FU alone in the previous study. Only those patients without liver involvement who were treated with high-dose CYC and 5-FU experienced prolonged survival.

A median survival of 35 weeks has been reported in a cooperative group trial for patients without liver involvement treated with radiation therapy and 5-FU (7). However, that trial was not controlled against patients treated with chemotherapy alone. In the current study, patients without liver involvement treated with a high-dose CYC and 5-FU combination had a median survival of 57 weeks. Therefore, studies comparing combination chemotherapy with radiation therapy plus chemotherapy could be justified in this disease.

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Fetal Sulfoglycoprotein Antigen and the Treatment of Gastric Cancer

Fetal sulfoglycoprotein antigen (FSA) can be detected in gastric aspirates by an immunodiffusion technique using rabbit FSA antiserum (1). Häkkinen and Viikari (2) have demonstrated that FSA is present in 96% of patients with gastric cancer and absent in 100% of normal young control subjects. In screening studies, FSA has been used successfully to detect early stages of gastric cancer (3).

The present study was undertaken to determine if FSA assay has value in predicting the efficacy of chemotherapy and supervoltage radiation therapy for inoperable gastric cancer. The results were compared with roentgenographic findings obtained by the hypotonic double-contrast technique providing good visualization of the gastric wall (4).

PATIENTS AND METHODS

Six patients with inoperable gastric cancer, proved by explorative laparotomy, were selected for analysis. Initial treatment usually consisted of ftorafur given orally (2.4 g daily for 15 days, repeated at 6-week intervals). If progression occurred, therapy was changed to iv infusions of 5-fluorouracil (1 g daily for 10 days) combined with CCNU (100 mg/m^2) or with supervoltage radiation therapy. The patients were followed for 6–12 months.

FSA was assayed at the beginning of treatment, at the end of treatment, once during the course of treatment, and once during the interval between each treatment, according to the method described by Häkkinen (1). The twice-absorbed samples were graded semiquantitatively as +, ++, +++, and –. Double-contrast roentgenographic examinations were performed at 2–3-month intervals, according to a technique described elsewhere (4,5), ensuring that identical projections were obtained in each consecutive study. The patients were analyzed three to seven times during the followup period.

RESULTS AND DISCUSSION

Table 1 shows the results obtained in individual patients. In two patients (Nos. 1 and 6) partial remission or stabilized disease was seen, as judged radiographically. These patients showed occasional trends of diminution or even disappearance of FSA from the gastric juice during the treatments. All other patients showed

TABLE 1.—Summary of patient characteristics and results

Patient No./age/sex	Followup time (mos)	Gross appearance of tumor	Histology	Treatment	Behavior of FSA	Roentgenographic findings
1/68/M	12	10 cm in diameter, cardia and fundus; retroperitoneal invasion	Poorly differentiated adenocarcinoma	Ftorafur, orally for 9 cycles	Disappearance during 1st cycle; diminution during 7th cycle	Decrease of intraluminal tumor, then polypoid tumor remained constant for 7 mos, then progressive infiltration of gastric wall
2/60/M	10	8 cm in diameter; invasion to pancreas	Highly differentiated adenocarcinoma, intestinal type	Ftorafur, orally for 2 cycles, then 5-fluorouracil, iv for 5 cycles	Varying values, occasionally negative at the start of medication but mostly positive (+ +)	Constant progression
3/66/M	7	Localized in fundus; retroperitoneal invasion; numerous local metastases	Poorly differentiated, diffuse type	Ftorafur, iv for 2 cycles, then CCNU orally with 5-fluorouracil, iv for 4 cycles	Constantly strongly positive (+ + or + + +)	Constant progression
4/59/F	6	4 cm in diameter, curvatura minor; liver metastases	Moderately differentiated adenocarcinoma	5-Fluorouracil, iv for 5 cycles	Constantly positive, varying irregularly between + and + + +	Constant progression
5/63/M	7	6 cm in diameter; invasion to pancreas and celiac axis; ulceration	Poorly differentiated, solid type	Ftorafur, orally for 3 cycles, then radiotherapy	Constantly positive, but disappearance during radiotherapy	Constant progression
6/39/M	8	Palliative resection of antral tumor; peritoneal carcinosis	Moderately differentiated adenocarcinoma, diffuse type	Ftorafur, iv for 4 cycles	Disappearance during 1st cycle, diminution during 2nd cycle, then positive (+ +) during subsequent cycles	Stabilized disease for 6 mos, then constant progression

radiographic progression of the cancer. FSA showed either irregular behavior or a strongly positive reaction throughout the observation period. In one patient (No. 5) FSA disappeared from the gastric juice during supervoltage radiation therapy. Only one patient (No. 1) showed a clear-cut decrease of the intraluminal portion of the tumor during ftorafur chemotherapy. Later, the radiographs showed a progressive increase of the rigid area of the gastric wall. Another patient (No. 6) showed no change in tumor size for 6 months but the tumor later increased in size. All other patients showed a constant increase of the tumor infiltration in the gastric wall.

The cancerous gastric mucosa in a dedifferentiated state shows gene expressions physiologically seen only during the fetal period. Our observations suggest that the FSA gene expression is changed only slightly as a result of anticancer chemotherapy or radiation. If small alterations were present, the semiquantitative method of FSA determination overlooked them. In the two patients with stabilized disease, occasional temporary diminution or disappearance of FSA was recorded during chemotherapy. The patients with constant progressive disease always showed an irregular or a strongly positive FSA reaction.

Major advances in roentgenographic diagnostic accuracy are based on the modern contrast suspensions, which are able to adhere to the gastric wall even under suboptimal conditions (4). The use of glucagon-induced relaxation in the double-contrast examination significantly improves the possibilities for evaluation of the extent of cancer growth (5).

While more effective chemotherapy for gastric cancer is likely to be developed in the future, it is important to have methods for evaluation of the effects of planned treatments as early as possible, in order to save patients from unpleasant side effects of ineffective treatments. FSA assay is a potential tool. Because it is presently only a semiquantitative method and can be used only in gastric

aspirates, it is not practical or reliable for followup studies. A quantitative radioimmunoassay for FSA and adaptation of this analysis for use in blood samples may prove more useful for followup purposes.

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Successful Re-induction Therapy for Acute Myelogenous Leukemia With Cytosine Arabinoside and Daunorubicin[1]

Therapy for acute myelogenous leukemia (AML) in relapse is a practical problem which increases in frequency as remission rates in AML improve. As this report indicates, the use of cytosine arabinoside (ARA-C) and daunorubicin (DNR) as re-induction therapy is a reasonable option for patients initially achieving a complete remission (CR) with these drugs.

Eleven patients were studied between August 1977 and February 1979. This included all patients seen at our institution with documented bone marrow relapse of AML after an initial CR was obtained using 100 mg/m^2/day of ARA-C iv by continuous infusion for 7 days and 45 mg/m^2/day of DNR for 3 days. Intensive consolidation therapy included ARA-C, DNR, cyclophosphamide, and 6-thioguanine in nine of the 11 patients; the other two patients relapsed while receiving 5-azacytidine consolidation therapy. For re-induction therapy, all of the 11 patients received ARA-C and DNR in doses identical to those used as induction therapy. Standard criteria for remission were employed.

Information regarding age, sex, duration of initial remission, and results of re-induction therapy are shown in table 1. Ten of 11 patients (91%) achieved a remission, and eight (73%) were CRs. Excluding the two patients who received marrow transplants following a second remission, the median duration of second remissions is 13 weeks and the median actuarial survival from the time of re-induction therapy is 31 weeks.

Our results suggest that in patients receiving ARA-C and DNR as induction therapy for AML, including those who were receiving maintenance ARA-C, the initial relapse does not necessarily indicate the emergence of a new drug-resistant tumor population. Although the duration of second remissions was less than the duration of first remissions (13 versus 46 weeks; $P < 0.03$), this may reflect the fact that second remissions were generally not consolidated and maintained as aggressively as first remissions. Although marrow tolerance might prevent such aggressive maintenance, one patient who has received further therapy with ARA-C and DNR has remained in remission for 61 weeks. The finding that these patients respond to ARA-C and DNR at the time of relapse also suggests that these drugs should be incorporated more intensively into maintenance regimens, since the best hope for the cure of AML probably depends on the achievement of durable initial CRs.

It was initially surprising that our re-induction rate compares favorably to the 60%–65% initial induction rate previously reported for this regimen (1,2). However, in giving re-induction therapy to patients who have already achieved one remission we are probably dealing with a selected population. We speculate that there may be a greater ability of the marrow to regenerate after chemotherapy in these patients (in contrast to the entire population of AML patients, including those who died during initial induction therapy). Alternatively, these patients might have a greater resistance to infection than other patients. Even though we cannot provide specific data regarding these points, it is true that ARA-C and DNR do produce higher remission rates in younger patients (1,3) and that the median age in our re-induction series, 35 years, is younger than the median age of the general AML population.

In view of the limited duration of the remissions achieved in this series, we cannot recommend ARA-C and DNR as the optimal therapy for patients relapsing with AML. Nevertheless, we believe that the high remission rate which we observed is important for several

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TABLE 1.—Response and survival of patients receiving re-induction chemotherapy

Patient No.	Age/sex	Duration of 1st remission (wks)	Response* to re-induction therapy	Duration of 2nd remission (wks)	Survival following re-induction therapy (wks)
1	22/F	48	CR	8	76
2	29/M	45	CR	*Received marrow transplant*	
3	29/F	46	CR	13	21
4	40/F	18	PR	4	8
5	62/M	66	Died	–	–
6	37/M	165	CR	61+	61+
7	30/F	56	CR	24	39
8	35/F	95	PR	*Received marrow transplant*	
9	64/M	25	CR	9	9
10	23/M	65	CR	14	27+
11	61/F	31	CR	20	26+

*CR = complete remission; PR = partial remission.

reasons. Any decision to switch therapy, including the institution of experimental agents, must consider the possibility that re-induction with agents which have been previously used will be successful. In addition, since therapeutic pessimism may lead to inadequate supportive care, demonstrating that second remissions can frequently be achieved with this approach may lead to more aggressive supportive care for these patients and to better results. Finally, if a policy is adopted to perform bone marrow transplants only in patients who are in remission, knowledge regarding the possibility of remission re-induction is essential for the development of a rational treatment strategy.

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Tamoxifen and Estrogen Hypercalcemia

Tamoxifen, a nonsteroidal antiestrogen, has recently been released in North America for use in the palliative treatment of disseminated breast cancer. Its action as an antiestrogen appears to be due to the blocking of circulating estrogens by its competitive binding to estrogen receptor binding sites in the cytoplasm (1). Estrogen receptor-tamoxifen complexes may then inhibit DNA synthesis after translocation to the nucleus as "false messenger units" (2).

There have been several reports of hypercalcemia associated with tamoxifen administration (2,3). Hypercalcemia has been documented in estrogen receptor-negative (4) and -positive (5) patients; it has been demonstrated that hypercalcemia occurs particularly in patients who have widespread skeletal metastases and a slightly raised pretreatment calcium level (6), and it has been suggested that tamoxifen may be re-administered with therapeutic benefit to patients experiencing early hypercalcemia (7). Caution has also been voiced in the attribution of hypercalcemia to tamoxifen without meeting strict criteria (8).

We have recently attempted the administration of tamoxifen to patients in whom severe estrogen-induced hypercalcemia had previously necessitated stopping estrogen therapy. The substitution of tamoxifen, despite mild hypercalcemia in one patient, was successful, and the patients are currently enjoying high-quality clinical remissions.

Patient 1

An 80-year-old woman presented with symptoms of fatigue, anorexia, and weight loss in December 1977. She was found to have a 3–4-cm breast lump in the right breast and was anemic. Biopsy showed adenocarcinoma. The marrow was tumor-infiltrated and roentgenograms showed extensive osteolytic and osteoblastic lesions.

She was given stilbestrol (5 mg twice daily), the serum calcium prior to starting estrogen being 9.7 mg/100 ml. One week later she was confused and disoriented, and complained of thirst and polyuria. She also complained

of increased bone pains. Her calcium was 12 mg/100 ml which rose rapidly over the next 24 hours to 20.6 mg/100 ml. She became stuporous and estrogens were stopped. She had a full recovery after 9 days of therapy with fluids, diuretics, steroids, and mithramycin. She subsequently received low-dose chemotherapy with reasonable control, until in October 1978 clinical symptoms, the appearance of skin nodules, and deteriorating bone scan and skeletal roentgenograms indicated progression of disease. She began receiving tamoxifen (10 mg twice daily orally) in the hospital and the serum calcium was measured daily. No hypercalcemia was documented and the patient was discharged after 10 days. The skin nodules regressed and roentgenograms showed sclerosis and healing of bone lesions; she was enjoying a high-quality remission at the time of her most recent assessment (June 1979).

Patient 2

A 67-year-old patient presented in 1973 with a 6-month history of a mass in the left breast. A modified radical mastectomy was performed; histologic examination showed an infiltrating ductal carcinoma with invasion of the axillary lymph nodes. Postoperative radiotherapy was given. In November 1976, she presented with anorexia and a weight loss of 30 pounds. Roentgenograms revealed osteoblastic metastases and in February 1976 she was given stilbestrol (5 mg twice daily orally). Four days later she came to the emergency department with severe nausea, vomiting, confusion, and disorientation. Her serum calcium was 17.6 mg/100 ml and she was treated with prednisone, Lasix, and saline. Over a period of 4 days her calcium fell to 8.8 mg/100 ml.

In view of the symptomatic hypercalcemia, the estrogens were stopped and she was given chemotherapy, which continued for a period of approximately 2½ years after which time bone scans and roentgenograms showed clear evidence of progression of disease.

In October 1978, she began receiving tamoxifen (10 mg twice daily orally); calcium was measured daily in the hospital. After 10 days of drug treatment she developed an asymptomatic hypercalcemia with a level of 12.5 mg/100 ml. She was given iv fluids, and 7 days later the calcium was within normal limits. The patient was well, free of pain, and responding to tamoxifen at the time of her last assessment (May 1979).

The clinical "flare" of bone pain associated with tamoxifen has been explained as "a transient estrogen-like activity." The hypercalcemia associated may presumably be explained similarly. It has recently been suggested that tamoxifen has a biphasic action (9), with estrogen agonist effects during initial administration but subsequent inhibitory effects at higher concentrations of the drug. From our experience in these two patients, who acted as their own "controls," it seems that the hypercalcemia associated with tamoxifen may be either less severe, as in patient 2, or may not occur at all, as in patient 1. If patients do have hypercalcemia this may be successfully managed in the hospital using a simple fluid and diuretic regimen, with the ultimate benefit of a clinical response.

In patients who have metastatic breast cancer previously treated with estrogens which were stopped because of severe symptomatic hypercalcemia, a trial of tamoxifen in the hospital is justified. This may induce a remission even if the patient has received combination chemotherapy for several years after attempted estrogen therapy.

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Prednisolone, Methotrexate, and 6-Mercaptopurine in the Treatment of Histiocytosis X

Patients with histiocytosis X have been reported to respond to most classes of chemotherapeutic agents. The results of two different multi-drug regimens modeled after schedules used in Hodgkin's disease have already been reported (1,2). The purpose of this study was to evaluate the efficacy of a combination of a corticosteroid and antimetabolites in histiocytosis X. Prednisolone, methotrexate, and 6-mercaptopurine have been combined with a vinca alkaloid with defined toxicity (vincristine) in the antileukemia regimen POMP (3); all drugs were given iv. In contrast to agents in previously studied

TABLE 1.—Response and toxicity

Patient No.	Response	Toxicity	Survival (mos)
1	Improvement	Severe neutropenia*	8+, active disease
	Progressive disease	*Pneumocystis carinii*	
2	Improvement	Mild thrombocytopenia,† severe neutropenia,* mucositis, elevated liver enzymes	9+, active disease
3	Progressive disease	Personality change	8+, active disease
4	Progressive disease	Glycosuria and ketonuria, moderate neutropenia‡	5+, active disease

*Severe neutropenia = absolute granulocyte count < 500/mm^3.
†Mild thrombocytopenia = platelet count 75,000–99,000/mm^3.
‡Moderate neutropenia = absolute granulocyte count 500–1000/mm^3.

combinations (1,2), these drugs are not known carcinogens.

Patients of any age with biopsy-proven histiocytosis X were eligible for the study; excluded were those for whom solitary eosinophilic granuloma was the sole evidence of disease. Patients treated previously with methotrexate or 6-mercaptopurine were not eligible.

Five-day courses were to be given every 2 weeks for 8 weeks with courses delayed until the absolute granulocyte count was > 1000/mm^3 and the platelet count was > 75,000/mm^3, unless these were attributable to disease. Methotrexate was given at a dose of 7.5 mg/m^2/day × 5 days by rapid iv push. 6-Mercaptopurine was given by iv drip at a dose of 500 mg/m^2/day × 5 days. Prednisolone was given by iv push at a dose of 1000 mg/m^2/day × 5 days.

The criteria for response were as previously described (1). Complete response was defined as the disappearance of all measurable lesions, including bone lesions, with no new lesions appearing. Partial response was defined as at least a 50% reduction in all measurable lesions. Improvement was defined as a < 50% reduction in measurable lesions.

The study was closed after the review of these patients since the only response was a minimal one and was accompanied by severe toxicity (table 1). Previous chemotherapeutic trials in poor-risk patients with histiocytosis X have achieved complete or partial response in 35% of patients (2). This study is the third attempt by the Southwest Oncology Group to evaluate combinations of known effective drugs. To date, none has shown any improvement over results achieved with component single agents in poor-risk patients.

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5-Fluorouracil in the Treatment of Acute Leukemia: A Southwest Oncology Group Study[1,2]

5-Fluorouracil (5-FU) and its 2′-deoxyuridine derivative (5-FUDR) have had limited trials in acute leukemia

[1]This study was performed by the Southwest Oncology Group and was supported in part by Public Health Service grants CA-03096 (J. J. Costanzi), CA-03389 (R. Gagliano), CA-22433 (C. A. Coltman, Jr), CA-16422 (J. N. Bickers), CA-16943 (Operations Office), and CA-12014 (Statistical Office) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare. In addition to the patients contributed by the authors, patients were also contributed by Drs. H. Hynes (University of Kansas Medical Center; CA-12644), J. Hewlett (Cleveland Clinic; CA-04919), and B. W. Amaral (Scott and White Clinic; CA-10187).

[2]The authors acknowledge P. Anderson for her technical assistance.

in adults, with most of the experience being in childhood leukemia (1–5). In general, these compounds have had limited usefulness in childhood leukemia. This overall lack of significant efficacy of 5-FU or 5-FUDR in childhood acute leukemia does not necessarily indicate that 5-FU would be ineffective in acute leukemia in adults.

Since a major goal of the treatment of acute leukemia is to produce a transient marrow aplasia, doses and scheduling of 5-FU which produce this response should be used. A large number of studies have consistently shown that a large daily iv bolus loading dose schedule of 5-FU, as originally described by Curreri et al (1), produces the most consistent marrow suppression compared to the weekly iv push or continuous iv infusion schedules (1). Therefore, 5-FU was used in a Southwest Oncology Group study for remission induction of acute leukemia in patients who have relapsed or who are not eligible for higher priority treatment programs.

MATERIALS AND METHODS

Seventeen patients with a bone marrow diagnosis of acute leukemia (myelocytic, monomyelocytic, or undifferentiated) in relapse were studied. The initial starting dose of 5-FU was 600 mg/m^2 by iv push daily for 7 days. After the 7-day course of chemotherapy, therapy was to be discontinued for 10 days. If recovery from the nadir of cytopenia was unusually prompt, the next treatment began earlier than Day 17. For all other patients a second course was started on Day 17. Patients who achieved a complete response continued to receive maintenance 5-FU at 21–28-day intervals, with dose escalation or de-escalation used to maintain platelet counts at > 50,000/ml^3 and granulocyte counts at > 1000/ml^3.

RESULTS

All 17 patients treated with this regimen were poor-risk patients, having received a median of four courses of various single-agent or combination chemotherapeutic regimens. Five of the 17 patients noted a good biologic response to 5-FU (table 1). One patient achieved a complete remission. She was a 40-year-old female with acute monomyelocytic leukemia who had had a 36-week remission with OAP (vincristine, cytosine arabinoside, and prednisone) followed by a 20-week remission with Adriamycin. Following these two regimens the patient had increasing disease with POMP (6-mercaptopurine, vincristine, methotrexate, and prednisone) and subsequently with 5-azacytidine. After one course of 5-FU therapy the patient became aplastic but her marrow promptly recovered, with the peripheral blood and marrow becoming normal. Her complete remission lasted 12

TABLE 1.—Characteristics of patients achieving a biologic effect

Patient age/sex	Type of leukemia*	Previous treatment,† type of response,‡ length of response (wks)	Length of 5-FU response (wks)	Comments
24/F	AMML	COAP, CR, 20 AD + OAP, CR, 12	6	Splenomegaly cleared; bone marrow cellularity decreased and platelet count rose
13/M	AMML	VCR + Pred, PR, 4 6-MP, PR, 12 OAP, PR, 20	3	Peripheral blood lost all blast cells; marrow became irreversibly aplastic
40/F	AMML	OAP, CR, 36 AD, CR, 20 POMP, ID 5-AZA, ID	12	Peripheral blood and marrow became normal
39/M	AUL	AD + OAP, CR, 24 POMP, CR, 8	8	Peripheral blood became normal; marrow blast cells decreased from 80% to 15%
21/M	AML	OAP, CR, 10 AD + OAP, NR POMP, ID	4	Peripheral blood lost all blast cells; marrow became irreversibly aplastic

*AMML = acute monomyelocytic leukemia; AUL = acute undifferentiated leukemia; AML = acute myelocytic leukemia.

†COAP = cyclophosphamide, vincristine, cytosine arabinoside, and prednisone; AD = Adriamycin; VCR = vincristine; Pred = prednisone; 6-MP = 6-mercaptopurine; POMP = 6-mercaptopurine, vincristine, methotrexate, and prednisone; 5-AZA = 5-azacytidine.

‡CR = complete remission; PR = partial remission; NR = no response; ID = increasing disease.

weeks. The patient subsequently redeveloped a leukemic marrow and died of sepsis. Table 1 shows the characteristics of the other four patients who had partial responses. Although the length of these responses was very short, it must be pointed out that all patients were extremely ill; two patients had active bacterial infections when therapy was started and two patients had aspergillosis. The other 12 patients had increasing disease and all died within 2 weeks of beginning 5-FU therapy.

Two of the 17 patients had no toxicity but four had moderate to life-threatening diarrhea and mucositis. One patient had severe leukopenia and thrombocytopenia and two patients had irreversible thrombocytopenia, leukopenia, and sepsis.

DISCUSSION

This study demonstrates that 5-FU can produce a positive biologic response in patients with acute leukemia. One of the 17 patients had a complete remission lasting 12 weeks. There were four partial remissions ranging from 3 to 8 weeks. All patients were heavily pretreated, having received a median of four courses of either a single agent or combination chemotherapy prior to receiving the 5-FU. Performance status in all patients was between 50 and 70 (Karnofsky scale). Two patients had no toxicity, four patients experienced moderate to severe diarrhea and mucositis, one patient had severe leukopenia and thrombocytopenia, and two patients had prolonged myelosuppression and sepsis. Although these toxic effects are to be expected with high iv bolus doses of 5-FU, the poor performance status of these patients probably contributed greatly to the morbidity and mortality from the toxicity.

From these data it can be concluded that 5-FU is active in the treatment of previously treated adults with acute leukemia.

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Nitrosourea-Associated Pulmonary Fibrosis[1]

Pulmonary fibrosis is a recently recognized but rare complication of nitrosourea therapy. The determinants of lung toxicity have not yet been characterized. This report documents one additional case and characterizes the clinical setting in which pulmonary fibrosis has been encountered.

CASE REPORT

A 10-year-old girl with a 3-month history of vomiting was found to have papilledema and radiologic evidence of a posterior fossa mass. Following subtotal resection of a cerebellar medulloblastoma, she was treated with craniospinal irradiation and adjuvant chemotherapy. The latter consisted of intrathecal methotrexate and iv vincristine given weekly for 5 weeks and dexamethasone given daily. Monthly injections of BCNU and vincristine were begun at 23 weeks. Because of positive spinal fluid cytology, intrathecal methotrexate was reinstituted at 45 weeks. Following six weekly injections, methotrexate was given once monthly. Sixty-three weeks after diagnosis, breathlessness was noted for the first time. A chest radiograph demonstrated bilateral hilar infiltrates, prompting discontinuation of methotrexate. The infiltrates cleared, only to recur when combined BCNU and methotrexate were resumed at 69 weeks. Thereafter, chemotherapy was discontinued. Over the ensuing year dyspnea worsened. An open lung biopsy at 119 weeks showed extensive pulmonary fibrosis. No infectious agent was identified. A course of prednisone effected modest symptomatic relief and improvement in lung volume (although a severe restrictive defect persisted), but

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TABLE 1.—Reported cases of nitrosourea-associated pulmonary fibrosis

Patient No. (ref No.)	Diagnosis	Age at diagnosis (yrs)	Nitroso-urea	Cumulative dose (mg/m^2)	Treatment duration* (mos)	Schedule (mg/m^2, interval)	Concomitant therapy
1 (7)	Astrocytoma	19	BCNU	1881	33	100, 3–4 wks	Cranial radiation
2 (8)	Medulloblastoma	2	BCNU	1550	26	100, 6 wks	Cranial radiation, vincristine, dexamethasone
3 (9)	Melanoma	43	BCNU	> 1060	> 22	150, 10 days	DTIC, hydroxyurea
4 (10)	Melanoma	45	Methyl-CCNU	2733	25	300, 6 wks	None
5 (11)	Ewing's sarcoma	14	BCNU	1000	4	50–150, 1 wk	Pulmonary radiation
6 (present report)	Medulloblastoma	10	BCNU	1228	11	75–150, 1 mo	Cranial radiation, intrathecal methotrexate, vincristine, dexamethasone

*Treatment duration in mos prior to onset of symptoms of pulmonary fibrosis.

at 130 weeks the patient developed severe respiratory insufficiency and died. The cumulative doses of BCNU and methotrexate were 1228 and 150 mg/m^2 respectively.

DISCUSSION

The potential for nitroso compounds to produce lung injury was recognized during preclinical trials. Pulmonary vascular damage was noted in mice given 1-methyl-3-nitro-1-nitrosoguanidine (1), and pulmonary edema occurred both in dogs and rhesus monkeys given the former agent or BCNU (2). A protracted decrease in the NADase activity of mouse lungs following BCNU exposure (3) has been proposed as a factor mediating tissue injury. In addition, the pharmacologic parallels between BCNU and the alkylating agents suggest a common basis for the pulmonary toxicity associated with BCNU, busulfan, and cyclophosphamide (4).

The patient described in this report developed pulmonary infiltrates followed by progressive fibrosis despite the early withdrawal of antitumor therapy. Although methotrexate has been incriminated in the pathogenesis of interstitial pneumonitis (5), it is unlikely that it played a major toxic role in our patient. The histopathologic features noted on the lung biopsy were not those usually seen with methotrexate-associated pneumonitis and the amount of drug given was small relative to that generally producing pneumonitis. Our patient received no more than 12 mg/week which was given by the intrathecal route with folinic acid protection. In the single reported case of pneumonitis following methotrexate given intrathecally, 10 mg/m^2 was injected every 48 hours for 20 days (6).

Selected features of biopsy-documented fibrosis attributed to nitrosourea therapy are summarized in table 1. It is noteworthy that the median age for the group (16 years) is well below that of patients with cancer. Relative youthfulness holds true within diagnostic categories: the ages of three patients with brain tumor and of two patients with malignant melanoma are below the medians for patients with these disorders. BCNU was held responsible for pulmonary fibrosis in five cases and methyl-CCNU in one case. All patients received a cumulative dose of $\geq$ 1000 mg/m^2 of the nitrosourea prior to the development of pulmonary symptoms. Duration of therapy was $\geq$ 11 months except in patient 5, in whom concomitant whole-lung irradiation (1200 rads) and BCNU administration were associated with pulmonary fibrosis within 4 months. The interval between doses of the nitrosourea varied from 1 to 6 weeks. There was no consistent adjunctive treatment. The predominant symptoms were those of pulmonary fibrosis, namely dyspnea and/or cough. Progressive pulmonary insufficiency was responsible for death in all except patient 4, whose death was due to progressive melanoma.

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VP-16-213 and Cyclophosphamide in Small Cell Lung Cancer: A Long-Term Followup Report

In 1977, we reported preliminary results on a phase II study of polychemotherapy and radiation therapy (XRT) for limited small cell carcinoma of the lung (1). Chemotherapy regimens were VP-16-213 plus cyclophosphamide (CYC), VP-16-213 plus Adriamycin (ADR), and CYC, ADR, and *cis*-dichlorodiammineplatinum(II) (*cis*-platin) (CAP). Each regimen was supplemented with local XRT. We report herein the long-term followup for this study, with particular attention to the > 3-year survivals observed in the VP-16-213 + CYC-treated patients.

METHODS AND MATERIALS

For extensive details of the characteristics of the study patients, staging procedures, categories of disease, criteria of response, stratification and randomization, and chemotherapy, readers are referred to our original report (1). All patients had small cell lung cancer limited to one hemithorax with or without ipsilateral supraclavicular fossa involvement.

The VP-16-213 + CYC regimen included VP-16-213, 70–75 mg/m^2 iv given on Days 1, 3, and 5, and CYC, 600–675 mg/m^2 iv given on Day 1. This cycle was repeated every 4–5 weeks. The VP-16-213 + ADR regimen included VP-16-213 as above plus ADR, 40–45 mg/m^2 iv on Day 1 repeated every 4–5 weeks. The CAP regimen included CYC, 400 mg/m^2 iv, ADR, 40 mg/m^2 iv, and *cis*-platin, 30–40 mg/m^2 iv, all given on Day 1 of a 4-week cycle.

A total of ten chemotherapy cycles were planned. Patients were re-evaluated 4 weeks after their first treatment. At that time 28 of 30 patients began receiving XRT. This consisted of 2000 rads in five fractions over 5–7 days using either a 4- or 10-MEV linear accelerator. Following a 3-week rest period, these patients received a second identical course of XRT. Details of the technique including spinal cord protection have been previously described (2). Two patients were not treated with XRT because of immediate evidence of disease progression.

RESULTS

Four of 30 patients in the original study population are alive. All four had been randomized to the VP-16-213 + CYC group. Following their first cycle of chemotherapy these four patients achieved one complete regression, two partial regressions, and one regression respectively. With further chemotherapy and XRT, these responses became three complete regressions and one regression. Recurrence was documented in one patient 10 months after completing six cycles of VP-16-213 + CYC. Complete regression occurred with local XRT and six cycles of ADR + *cis*-platin. These four patients treated with VP-16-213 + CYC are alive and disease-free at 38, 38, 49, and 41 months from the start of treatment and 28, 29, 30, and 32 months from the completion of their treatment. Table 1 compares the responses and survivals of the patients in the three study groups.

Overall toxicity in these four patients was similar to that seen in the group receiving VP-16-213 + CYC as a whole. These patients had only mild gastrointestinal toxicity. Mild neutropenia (wbc nadir, 2200/mm^3) occurred in two of the four patients at some time during their course of treatment. Two patients had no neutropenia, ie, no wbc counts < 4000/mm^3. None of the patients developed thrombocytopenia, ie, platelet count nadir < 100,000/mm^3.

Although not present in these four patients, central nervous system (CNS) metastases were subsequently diagnosed in five of the 30 study patients (17%). In the VP-16-213 + CYC group, two of 12 patients (17%) developed such metastases, whereas the rate of CNS involvement in the CAP-treated group was 36% (three of eight patients). No CNS metastases developed in the ten patients treated with VP-16-213 + ADR.

DISCUSSION

The overall regression rates and median survivals for

TABLE 1.—Response after 1st cycle of chemotherapy, response after chemotherapy + XRT, and survival by treatment group*

Treatment groups	No. of patients								Median survival (days)
	Best response after 1st cycle				Best response after XRT + chemotherapy				
	CR	PR	Regression	Stabilization	CR	PR	Regression	Stabilization	
VP-16-213 + CYC	1	5	3	3	4	2	5	1	383
VP-16-213 + ADR	0	2	6	1	3	1	5	0	387
CAP	0	4	2	2	3	2	3	0	269

*CR = complete regression; PR = partial regression.

patients receiving VP-16-213 + CYC, VP-16-213 + ADR, and CAP with XRT are comparable to those of other chemotherapy + XRT regimens as reviewed by Broder et al (3). Long-term survival of patients with limited small cell carcinoma of the lung now appears possible using combined modality treatment (4). Four of 12 patients treated with VP-16-213 + CYC have accomplished this with minimal toxicity.

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ANNOUNCEMENTS

2nd World Conference on Lung Cancer

Copenhagen/Malmö, June 9–13, 1980

The 2nd World Conference on Lung Cancer to be held in Copenhagen/Malmö, June 9–13, 1980, is sponsored by the International Association for the Study of Lung Cancer. The conference will present a succinct evaluation of current knowledge related to prevention, diagnosis, and treatment of lung cancer. All of the laboratory and clinical disciplines concerned with lung cancer will be represented in formal meetings, round-table discussions, workshops, scientific and technical exhibitions, and demonstrations on lung cancer. Topics to be covered include epidemiology, early diagnosis and staging, pathology, cellular kinetics, tumor markers, and all modalities of treatment such as surgery, radiotherapy, chemotherapy, and immunotherapy.

Further information about the conference may be obtained from: **DIS Congress Service, Linde Alle 48, DK-2720 Vanløse, Denmark (telephone 01-11 00 44), or from Secretary General, Heine H. Hansen, MD, Finsen Institute, 49, Strandboulevarden, DK-2100 Copenhagen, Denmark (telephone 01-26 08 50, extension 256 or 266).**

Medical Oncology Fellowship

A 1- or 2-year board-approved clinical fellowship program in Medical Oncology, affiliated with the University of Nevada School of Medical Sciences, is available in Las Vegas, Nevada, beginning July 1, 1980. One year of internship and 2 years of medical residency are required. The program is affiliated with the Southwest Oncology Group. The stipend is $15,000. ***For further information contact:*** **Joseph M. Quagliana, MD, 3201 S Maryland Pkwy, Suite 500, Las Vegas, NV 89109, telephone: (702) 733-6488 and 733-9421.**

Seminars on Oncology at Roswell Park Memorial Institute

Buffalo, New York

The Roswell Park Memorial Institute (RPMI) will hold the following seminars: "Complications in Cancer Patients: Paraneoplastic Syndromes"—Dr. Julian L. Ambrus, Chairperson (*February 8, 1980*); "Nutrition and Cancer"—Dr. Harold Douglass, Chairperson (for physicians, nurse practitioners, nurses, and allied health professionals and dietitians) (*February 28, 1980*); "Bone and Soft Tissue Malignancy"—Dr. E. Douglas Holyoke, Chairperson, sponsored by Department of Surgical Oncology (*March 7, 1980*); and "Cancer Prevention and Detection: Update for the Community"—Drs. Lazar and Curtis Mettlin, Chairpersons, sponsored by the RPMI and the Department of Family Practice, Millard Fillmore Hospital, Buffalo, NY (*March 20, 1980*).

These seminars qualify for Category I credit toward the American Medical Association Physician's Recognition Award, prescribed credit by the American Academy of Family Physicians, and Category 2-D credit by the American Osteopathic Association. There is a $25.00 registration fee. ***For further information contact:*** **Gayle Bersani, RN, Cancer Control Coordinator, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263 (telephone: 716-845-4406).**

5th Annual Symposium on Advances in Cancer Treatment Research

Baltimore, Maryland, March 6–8, 1980

The University of Maryland School of Medicine's Program of Continuing Education is presenting the 5th Annual Symposium on Advances in Cancer Treatment Research to be held at the Cross Keys Inn, Baltimore, Maryland, March 6–8, 1980. ***For additional information contact:*** Program of Continuing Education, University of Maryland School of Medicine, 10 S Pine St, Rm 300, Baltimore, MD 21201 (telephone: 301-528-3956).

Research Conference on Head and Neck Oncology

Bethesda, Maryland, September 8–10, 1980

The Division of Cancer Treatment of the National Cancer Institute is sponsoring a multidisciplinary Research Conference on Head and Neck Oncology to be held in Bethesda, Maryland, September 8–10, 1980. The purpose of the conference is to provide a forum for the discussion of recent experimental and clinical research in head and neck cancer and to assess areas of research which should have high priority for future development.

Participation will be limited to persons actively engaged in either experimental or clinical research. Conference topics will include recent clinical trials, new diagnostic and therapeutic approaches employing surgery, radiation therapy, chemotherapy, immune modulation, or other investigational modalities, and laboratory investigations dealing with immunology, virology, cell culture, and animal tumor-model development. Conference participants may elect to present recent research or research in progress or to participate in discussions. ***Interested investigators should contact*** Gregory T. Wolf, MD, Special Assistant for Surgical Oncology, Landow Bldg, Rm 8C17, National Cancer Institute, Bethesda, MD 20205, telephone (301) 496-4844.

15th Annual San Francisco Cancer Symposium on "The Pharmaceutical Aspects of Cancer Care"

San Francisco, California, March 15–16, 1980

The West Coast Cancer Foundation, in association with the American Cancer Society, is sponsoring the 15th Annual San Francisco Cancer Symposium on "The Pharmaceutical Aspects of Cancer Care" to be held at the Hyatt on Union Square in San Francisco, California, March 15–16, 1980. (Continuing education credit has been applied for.) ***For further information contact:*** West Coast Cancer Foundation, 50 Francisco St, Suite 200, San Francisco, CA 94133 (telephone 415-981-4590).

Symposium on "Hormone Manipulation in the Therapy of Human Malignant Disease"

Chicago, Illinois, April 15–16, 1980

The Section of Medical Oncology of the Rush Medical College and the Rush Cancer Center will sponsor an international symposium on "Hormone Manipulation in the Therapy of Human Malignant Disease" in Chicago, Illinois, April 15–16, 1980. The symposium will be held as a special tribute to Dr. Samuel G. Taylor III, one of the most distinguished oncologists in the US and the first director of the Section of Medical Oncology at Rush Medical College. An outstanding local, national, and international faculty will contribute to the program. ***For further information contact:*** Harold A. Paul, MD, Associate Dean, Office of Continuing Education, Rush Medical College, 600 S Paulina, Chicago, IL 60612 (telephone 312-942-6917).

Cancer Research Fellowships

Starting July 1, 1980, Cancer Research Fellowship positions will be available at the Comprehensive Cancer Center for the State of Florida, Miami, for training in areas of basic and clinical research (carcinogenesis, molecular and cellular biology, cellular proliferation, differentiation, pharmacokinetics, experimental chemotherapy, and radiotherapy). The program is designed for MDs with 2 years of previous training who plan an academic career in medicine. US citizens and permanent residents should send inquiries and curricula vitae *by February 1, 1980,* to: Francisco Tejada, MD, Department of Oncology, University of Miami School of Medicine, PO Box 016960 (D8-4), Miami, FL 33101.

CORRECTIONS

Several errors appear in the article "Mode of DNA Binding of *cis*-Platinum(II) Antitumor Drugs: A Base Sequence-Dependent Mechanism is Proposed" by Asher D. Kelman and Henry J. Peresie which appeared in the September/October issue of *Cancer Treatment Reports*. Figure 2 refers to deoxynucleosides. The prefix "deoxy" should appear before each base and the nucleosides are, hence, "deoxycytidine," "deoxyguanosine," "deoxythymidine," and "deoxyadenosine." On page 1447 (right column, line 12), the compound mentioned should be spelled "dichloroethylenediamineplatinum(II)." The second line in the last paragraph on page 1447 [beginning "Kong and Theophanides (7). . . ."] should read "*coupling* by nuclear magnetic resonance spectroscopy to." Line 7 of the first full paragraph on page 1449 should read "with N(3) of *cytosine*. We find these studies difficult to." Line 8 of the second full paragraph in the right column of page 1450 should read "tion *at* a platinum:base phosphate molar ratio of only." The full page numbers for reference 3 should be 1–827, and the correct journal name in reference 14 should be *Accounts of Chemical Research*.

The editorial staff of *Cancer Treatment Reports* wishes to point out three errors which appear in the Abstracts From the International Congress on Hormones and Cancer (Vol. 63, No. 7, July 1979). The sentence beginning on line 12 of abstract 101 should read as follows: "Scatchard analysis of the data indicated that the decrease in glucagon binding was due to a decrease in binding *capacity, whereas the decrease in insulin binding was due to a decrease in binding* affinity." The italicized words were omitted from the version submitted to the editorial office. In line 26 of abstract 99 the first word should be "Astrup." Also, the last sentence in abstract 99, excluding the acknowledgment line, should read as follows: "Our demonstration of high activities in human breast tumor cytosols prepared for receptor assay suggests the relevance of clinical tests *of the concept that steroid effects on human tumors may be mediated* by steroidal modulation of the synthesis, secretion and/or inhibition of plasminogen activator." The italicized words were omitted from the version submitted to the editorial office.

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